GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND
VARIATION

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CORRIGENDA

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Page 134, Key to Charts, line 4, for "on known facts concerning individual" read "no known facts concerning individual."

Page 155, formula, line 6, for "$p = \sqrt{\frac{AB-2Ab}{AB-Ab}}$" read "$p = \sqrt{\frac{AB-2Ab}{AB+Ab}}$"

Page 183, line 10, for "$bb^{def}/y^{bb}/$" read "$bb^{def}/Y^{bb}/$."

Page 185, line 14, for "$y\ sl^2\ bb^{def}/bb$" read "$y\ sl^2\ bb^{def}/y\ bb$."

Page 185, lines 14, 15, and 16, for "$y\ sl^2\ bb^{def}/bb/$" read "$y\ sl^2\ bb^{def}/y\ bb/$."

Page 387, Literature Cited, line 3, for "Genetics 18: 355–366" read "Genetics 18: 335–366."

H. Nilsen-Elde

NILS HERMAN NILSSON-EHLE

(FRONTISPIECE)

NILS HERMAN NILSSON-EHLE was born in 1873 at Skurup, Sweden. He received his scholastic training at LUND, and in 1909 received the doctor's degree from that institution. In 1910 he was appointed Professor of Botany at LUND, and in 1917, Professor of Genetics. His work has covered a wide field, but his most important contributions have been in the field of cereal genetics.

During his earlier years Professor NILSSON-EHLE was interested in problems of plant variation and distribution. He was a member of a botanical expedition to Eastern Siberia in 1898, and later visited Denmark, England, France, and Germany. Plant hybrids attracted his attention at the beginning of his scientific career, and among his earlier works are descriptions of hybrids of Salix species and different forms of Carex.

In 1909 Professor NILSSON-EHLE began his important work on cereal genetics with his contribution *Kreuzungsuntersuchungen an Hafer und Weizen*. In this paper he showed that certain characters, especially grain color in wheat, may be dependent on one, two, or three factors, and that genetic ratios of 3:1, 15:1, or 63:1 may be obtained in second generation hybrids, depending on the number of genetic factors involved. This work was followed by work on inheritance of various morphological characters in cereals, the genetics of disease resistance and winter-hardiness, and a genetic analysis of various physiological characters. In recent years considerable work has been done on the genetic behavior of speltoid mutations in Triticum.

Professor NILSSON-EHLE has always been interested in the practical application of genetics to agriculture and has produced many new types of wheat and oats which have proved to be more disease-resistant and more productive than the older varieties. His work is an excellent example of a combination of theoretical genetics and practical plant breeding.

GENE NUMBER, KIND, AND SIZE IN DROSOPHILA

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The Rockefeller Institute for Medical Research, Princeton, New Jersey

Received July 11, 1932

The literature on X-ray effects contains two fundamental ideas which, if combined, should offer a better understanding of the physical properties of the gene on the one hand, and of the mechanism by which X-rays affect living tissue on the other. The first of these ideas is due to CROWTHER. He interpreted the rates at which living cells are killed by X-rays as due to the destruction of a vital center within each of these cells. The second idea comes from MULLER'S demonstration that X-rays impinging on chromatin will produce new gene mutations which, in turn, lead to new heritable characters. In order that the problem may be sharply defined, we shall confine the discussion to data bearing upon the kind and size of the genes present in an animal organism. The approach lies in a knowledge of the physical characteristics of X-rays in relation to their biological effects and to the rates at which these alterations are produced. This paper presents an analysis of this problem on data obtained from the treatment of Drosophila sperm with long wave-length X-rays of known energy. Sperm of the wild-type were chosen since they represent concentrated chromatin whose full effects under normal conditions are better understood than those of any other material.

MATERIALS AND METHODS

The experiment utilizing the copper X-ray was performed first. Wild-type males and females taken from a stock which had been kept and studied in the laboratory for many years were mated. For the first cultures, 4 or 5 pairs were used to a bottle. This practice proved to be undesirable as the resulting progeny were crowded and somewhat reduced in size. In later work only pair mating was practiced. The male progeny, about 30 hours old, were placed in Petri dishes 1 inch in diameter, about $\frac{1}{8}$ of an inch deep, and covered with silk bolting cloth. These dishes and their contained males were placed in the X-ray beam from 30 to 240 seconds to give the requisite X-ray dosage. (The X-ray exposures in these experiments were made in the laboratory of DR. RALPH W. G. WYCKOFF. The writers are indebted to him for the use of his apparatus, for calculation of the X-ray ionization values, and for his advice and counsel.)

The previously described gas tube (WYCKOFF and LAGSDIN 1930), equipped with targets of different metals, has been used to obtain the intense beams of X-rays needed for these experiments. These beams con-

sisted mainly of the K lines of copper and chromium, depending on the metal which was used as the target. Because of the loss of intensity consequent upon filtration and the desirability of having the data with the different radiations as comparable as possible, no attempt has been made to render the beams in any of these experiments more nearly monochromatic. Other data on filtered and unfiltered copper rays indicate, however, that only a moderate error is introduced by this slight heterogeneity.

The current through the X-ray tube during the irradiations was held constant at 4 milliamperes. The peak voltage was 34 K.V. as measured with a 12.5 cm sphere gap.

With the soft rays from copper and chromium it is necessary to make a correction for the amount absorbed in the layer of air between the surface of introduction and the volume giving the ionization current. The length of this air column was 3.2 cm. Taking the absorption coefficient (KAYE 1923) of Cu K radiation as $\mu/P = 8.43$ and of Cr K as 28.0, calculation shows that the measured ionizations at the irradiated surfaces due to the X-rays are 97 percent for Cu K and 89 percent for Cr K. Introducing this absorption correction and transferring to standard air conditions, the measured air ionizations of the beams used in the experiments are expressed as the saturation currents due to the ions produced in a cube 1 cm on its edge.

Except for irradiation, all males in any one experiment were treated alike. The control and irradiated males were mated singly to females having the composition scute, Bar, small wing, vermilion, tan ($=s_c$ B s_m v t) in one sex chromosome and white miniature Beadex ($=w$ m B_z) in the other. The scute Bar-chromosome prevents most of the crossing over and carries a recessive lethal effect. Complete counts of the progeny were made for these matings over a period of 8 days for copper and 10 days for chromium. (The breeding technique for the males irradiated with copper was somewhat different from that of the males irradiated with chromium. A copper irradiated male was bred to a single female, the pair being left in the culture bottle 8 days and then transferred to a second bottle for another period of 8 days. The average count of the progeny in the first bottle was determined and utilized as the number of surviving sperm for each dose. A study of the two bottles showed that the relative result was essentially the same but that the numbers of individuals found in the second bottle were much reduced as compared with the progeny from the first. In view o these facts, it was decided to leave the chromium irradiated pairs in the bottles for the full ten days and to omit the second bottle test. The differences in the absolute numbers of progeny for the survival curves of the copper and chromium series are accounted for on this basis.) After hatching commenced the Bar females were mated singly to w m B_z stock

males. The progeny of these matings were then examined for recessive sex-linked lethals or visible mutations which might have been produced. If mutations were discovered further breeding experiments were made to locate the position of these changes within the chromosomes. The general scheme of the matings is the familiar *ClB* type as shown in figure 1.

Four types of information are determined from these matings:

1. The first noticeable effect of X-ray treatment of any organism is the sterility of this organism when it is bred to those of normal fertility. This sterility in the case of males is attributable to some alteration pro-

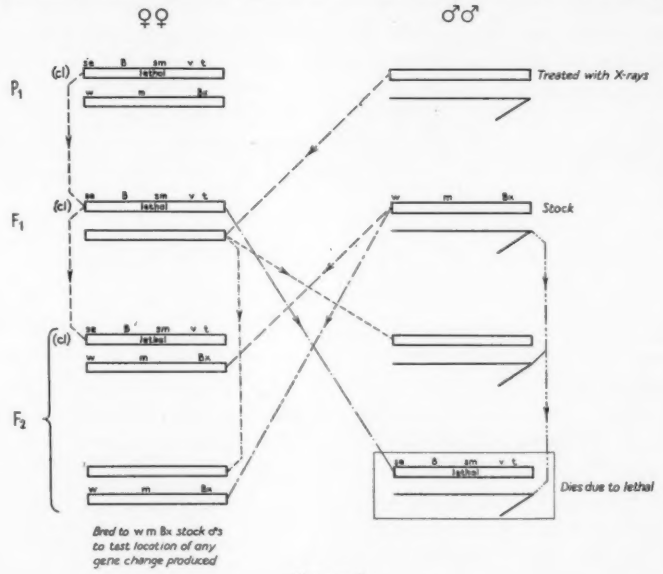

FIGURE 1.

duced within the sperm by the treatment. When such altered sperm fertilize normal eggs the embryos from these eggs die without becoming adult. The gene changes of this type produced by the X-rays may be looked upon as dominant lethals. These dominant lethals lower the number of adults surviving from matings sired by X-rayed males as contrasted with those sired by untreated males.

2. If one of these dominant lethals happens to be produced within the sex chromosome the lethal not only lowers the survival rate in this culture but it also lowers the number of females in relation to the number of males, since only females normally receive an X-rayed sex chromosome. This alteration in the sex ratio is consequently another direct measure of the number of dominant lethals produced by the X-rays.

3. The third class of data consists of the recessive lethals which are produced in the sex chromosome. These lethals manifest themselves by the fact that any F_1 female carrying such a lethal will have only female progeny. As the females containing the lethal do not die they may be bred for further testing the gene mutation and for locating its position within the chromosome.

4. A fourth group of information was obtained from the numbers of matings exhibiting visible mutations. These cases were analyzed for dominant or recessive sex-linked mutants and dominant autosomal mutations.

Data were collected on these four types of information for 8 durations of X-ray and for the two classes of rays, copper and chromium.

METHODS OF ANALYSIS

The survival curves measuring the dominant lethal mutations produced by the X-rays for the different dosages and wave lengths are presented in table 1.

TABLE 1

Survival curves for Drosophila sperm tabulated against time of exposure to X-ray and total X-ray exposure.

TIME IN SECONDS	COPPER SURVIVAL		SEX RATIO	TIME IN SECONDS	CHROMIUM SURVIVAL		SEX RATIO	e.s.u.
	NUMBER	RATIO			NUMBER	RATIO		
0	107.6±10.2	1.000	1.000±0.017	0	135.2±11.6	1.000	1.000±0.037	0
30	46.4± 8.7	0.431	0.960±0.029	37	69.0± 6.7	0.511	0.965±0.029	4460
60	18.9± 2.9	0.175	0.938±0.036	72	44.7± 3.0	0.330	0.909±0.027	8920
90	13.5± 2.9	0.126	0.867±0.042	111	17.2± 1.6	0.127	0.807±0.039	13380
120	7.4± 1.3	0.069	0.927±0.052	148	5.3± 0.6	0.039	0.792±0.064	17840
150	..	..	..	185	4.8± 1.1	0.036	0.934±0.081	22300
180	1.4± 0.3	0.013	0.779±0.067	222	1.3± 0.3	0.009	0.752±0.100	26760
210	.6± 0.1	0.005	0.898±0.108	259	0.9± 0.1	0.006	0.685±0.090	31220
240	.4± 0.1	0.004	0.654±0.157	..	..	..	..	35680
Ionization per sec./cm² in 1 cm of air			148.7				120.6	

The survival ratios of this table when plotted on semi-logarithmic scale have certain outstanding characteristics. The raw data form a straight line of constant slope without any lag period. If we think of the X-rays as a stream of bullets shooting at random at a target, this type of curve would be generated by the condition that one hit on the vital spot of the target would kill. This vital spot may be in one piece or may be subdivided into many parts scattered throughout the target, any one of which may be hit, the total area of these smaller spots equaling the entire area of the vital spot.

Sperm may be regarded as targets composed of compact bundles of

chromatin within which the genes are embedded, one gene following another as beads do on a string. Some of these genes are vital genes in the sense that alterations in their structure produce lethals which eventually cause the death of the organism. The death resulting from such changes probably occurs in Drosophila in the diploid development since it was possible to show that (1) females bred to X-rayed males contained sperm in numbers comparable to those bred to untreated males, (2) hatched eggs sired by X-rayed males died at a greater rate than those sired by normal parents, and (3) sperm and eggs carrying recessive lethal genes are not perceptibly decreased in numbers.

The vital genes which X-rays change to lethal factors are distributed approximately at random within the functional chromatin as shown by these experiments. By functional chromatin is meant chromatin carrying genes, as differentiated from that of the Y chromosome and the right-hand end of the X chromosome which, up to the present, and in spite of extensive work on Drosophila, has not been shown to carry genes in a manner comparable with the other chromatin material.

The vital genes are of two classes, those which produce their effect in haploid condition, or to make alternative classes, dominant vital genes *versus* recessive lethal genes, and those which cause the characteristic reaction only in diploid, recessive vital genes *versus* dominant lethal genes. The deaths brought about by the X-rays as shown in table 1 may be regarded as due to the rays hitting one of these vital spots with the consequent production of dominant lethal genes.

When an X-ray strikes matter it may be absorbed or it may pass through without effect. If the ray is absorbed it gives up one or more of its quanta with the coincident production of high speed electrons. These electrons in turn give rise to secondary electrons. The volume wherein these changes take place is small. This fact, together with the random distribution of the quantum absorptions over the chromatin, presents the chance element necessary to account for the experimental results.

The mathematical analysis of the set of conditions outlined above has been presented by several investigators (CROWTHER 1926). It leads to the equation

$$\text{survival} = \frac{\begin{matrix}\text{observed survival}\\ \text{after treatment}\end{matrix}}{\begin{matrix}\text{initial survival}\\ \text{without treatment}\end{matrix}} = e^{-ant}$$

where a is the probability of one shot hitting one of these vital spots, n is the number of shots in unit time, and t is the time of exposure. The consequences of this process may be visualized from a typical calculation.

In our experiment with copper radiation 148.7 electrostatic units were given per sec./cm^2 in 1 cm of air.

The number of ion pairs per sec. per cm^3 at the position of the irradiated sperm is consequently 148.7$\times$electrons in one electrostatic unit or 0.21 $\times 10^{10}$ ion pairs/sec. $= 3.12 \times 10^{11}$ ion pairs.sec./cm.^{3}

For material composed of atoms of low atomic weight these absorption coefficients vary directly with the density of the substance. If this conclusion is applied to chromatin, an assumption which cannot be far wrong, the amount of absorption per sperm head is $\frac{3.12\times10^{11}\times1\times10^{-12}\times1.00}{1.00\times1.165\times10^{-3}}$ $= 267$ ion pairs/sec./sperm. Since direct measurement of the dimensions of the sperm head are 7.36×10^{-4} cm long and 0.368×10^{-4} cm wide, hence—considering the sperm head a rectangular block of chromatin—the volume is 1.0×10^{-12} cm^3. The density of chromatin is assumed to be 1.00 and the density of the air at the temperature used is 1.165×10^{-3}.

In order to obtain the number of X-ray quanta absorbed per second it is necessary to know how many ions are liberated by one quantum absorbed in air. The best available measurements (KULENKAMPFF 1926) show that X-rays of the quality used require about 35 volts for each electron pair. The voltage equivalent of the K$-\alpha$ lines of copper follows from the quantum relation Voltage (in K.V.) $= 12.34$/wave length of copper X-rays (1.537) $= 8.029$ K.V.

The number of ion pairs arising through the absorption of one quantum of Cu K$-\alpha$ X-rays is $\frac{8{,}029}{35} = 229$. The average number of quantum absorptions per second is $\frac{267}{229} = 1.163$.

The average number of absorptions which actually produced death was determined from the experimental data by fitting the curve previously derived, survival $= e^{-at}$, to the actual data by the method of least squares. The resulting curve was $Y = e^{-0.024t}$.

If the sperm were entirely composed of vital recessive genes then every hit would be expected to kill, or the rate of killing with time should be 1.163 per second. The exponent 0.024 per second for the actual data shows that this is not the case. The chance of hitting this vital volume within the sperm clearly decreases as its volume relative to the whole sperm becomes less. The theoretical absorption 1.163 per second represents the whole volume. The effective absorption 0.024 represents the vital volume. Their ratio, 1:0.021, represents the whole volume compared with this vital volume. In other words, the normal sperm area has 21 thousandths of its volume composed of vital recessive genes.

In these calculations and those which follow, extensive use is made of the data on the size of the sperm and the chromosomes composing them. These measurements were obtained from material fixed in modified Bouin and stained with iron haematoxylin. Suitable sperm which were separate and distinct from each other were photographed at 770 diameters. The photographs were then made up on lantern slides and projected on a screen. The sperm were drawn from the projected images and the magnitude of the enlargement determined. The average size of the sperm head was 7.36×10^{-4} cm in length and 0.368×10^{-4} cm in breadth. The size of the individual chromosomes within the sperm head was determined from the oögonial divisions. The technic of fixation staining and enlargement was the same as that described for the sperm save that the initial magnification of the photograph was 2500 diameters. One of the division figures utilized contained besides the normal female complex a Y chromosome. The measurements of the different chromosomes are:

Sex or X chromosome	Length 1.56×10^{-4}cm	Average breadth 0.34×10^{-4}cm	Area 5.27×10^{-9}cm^2
II chromosome	Length 2.21×10^{-4}cm	Average breadth 0.33×10^{-4}cm	Area 7.22×10^{-9}cm^2
III chromosome	Length 2.80×10^{-4}cm	Average breadth 0.34×10^{-4}cm	Area 9.71×10^{-9}cm^2
IV chromosome	Length 0.28×10^{-4}cm	Average breadth 0.26×10^{-4}cm	Area 0.58×10^{-9}cm^2
Y chromosome	Length 1.85×10^{-4}cm	Average breadth 0.30×10^{-4}cm	Area 5.61×10^{-9}cm^2
Total haploid size with X chromosome	Length 6.85×10^{-4}cm	Average breadth 0.33×10^{-4}cm	Area 22.78×10^{-9}cm^2
Total haploid size with Y chromosome	Length 7.14×10^{-4}cm	Average breadth 0.32×10^{-4}cm	Area 23.12×10^{-9}cm^2

Figure 2 shows a sample photograph of the sperm used in obtaining these measurements. Figure 3 shows one of the metaphase oögonial figures, containing a Y chromosome measured for the size of its chromosomes.

While somewhat aside from the problem under discussion, the comparison of the measurements of the sperm head with those of the condensed metaphase chromosomes is significant from the viewpoint of the mechanism by which these chromosomes may be condensed into the sperm head. The maximum breadth of the sperm head represents the maximum breadth which a chromosome assumes if the sperm is to be one chromosome wide. This breadth is 0.368×10^{-4} cm or somewhat smaller. The breadth of each of the chromosomes is approximately the same as this figure, 0.33×10^{-4} cm. If chromosomes reach the stage of extreme condensation at the metaphase, then these results show that they must lie end to end in the sperm head. The total length of the chromosomes under these conditions should be the same as the length of the chromatin in the sperm head. The length of the sperm head is 7.36×10^{-4} cm, although this length is again probably somewhat large. The total length of the haploid chromosomes is 6.85×10^{-4} with the X, or 7.14×10^{-4} cm with the Y.

The result therefore corresponds with the view that the chromosomes lie end to end in the Drosophila sperm. These measurements on fixed tissue, while somewhat less than they would be if taken from living tissue are of the same order of magnitude.

The sex ratios in the second group of data were determined from the relation ♀/2♂ since the presence of the lethal in the *ClB* test females reduces the males to half their true number. These sex ratios were then

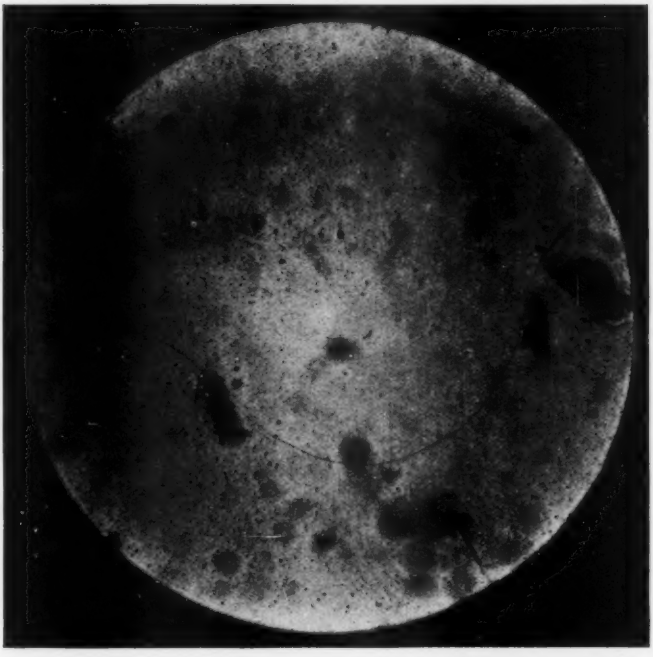

FIGURE 2.—Drosophila sperm taken from female. The tail of this sperm is at least three times as long as shown, and quite possibly longer since it may have been broken in handling the material. The acrosome, cytoplasmic sheath, etc. of the sperm head is stained in this preparation. Magnification 770.

adjusted to 100 as the ratio for the untreated class. The analyses of the sex ratio data were entirely similar to those for the survival curves of the whole organism as given above.

The recessive lethals could be tested and placed in their relative positions within the chromosome. The presence of one recessive lethal in general had no influence on the presence of another. It was therefore possible to determine whether or not a chromosome had more than one

lethal. This fact makes it possible to treat the data in two ways, leading to the same general conclusion.

If a is the chance of one shot hitting the target and n the number of

FIGURE 3.—Drosophila chromosome plate showing diploid group plus an extra Y. Magnification 2500.

shots in unit time, then the chance in a given number of units is $Y = ant$ instead of e^{-ant} as is the case when one hit makes it impossible to distinguish another hit on the target. The rate of change is again an however.

This curve is a summation of hits, chromosomes having two recessive lethals being counted twice, those with three being counted three times, etc. Obviously this method has real difficulties when it comes to determining the presence of two or more lethals closely associated in the chromosome.

The data may also be treated exactly the same as those for the dominant lethals to obviate this difficulty. The equation for the chromosomes which show no recessive mutations, or what might be looked upon as the survivors, becomes

$$Y = \frac{\text{observed chromosomes lacking recessive lethals after treatment}}{\text{initial chromosomes lacking recessive lethals before treatment}} = e^{-ant}$$

This curve is the curve which HANSON and HEYS (1929) should have applied to their data since their technique shows only the proportion of chromosomes which had no lethal mutations and not the total number of lethal mutations per given number of chromosomes. The analysis of their beautifully regular data supports this view as the curve e^{-ant} fitted to the proportion of the chromosomes which escape being hit fits the material more accurately than the curve $Y = ant$ worked out from the data as they present them.

EXPERIMENTAL DATA

Table 1 presents the data on the survival rates of sperm irradiated with increasing amounts of either copper or chromium X-ray. These data are plotted in figures 4 and 5. The middle bar for each irradiation gives the survival value attained in the experiment. The length of the heavy line shows the probable error of this determination. The data are plotted on semi-logarithmic scale since such a plot presents the curve $Y = e^{-ant}$ as a straight line. The equations for the survival curves for the copper irradiation are $Y = e^{-0.0244t}$ or $Y = e^{-0.0_3164e.s.u.}$ depending whether time of exposure in seconds or electrostatic units of irradiation are used as the unit of measure.

The survival curves for the sperm exposed to irradiation from chromium are $Y = e^{-0.0198t}$ or $Y = e^{-0.0_3164e.s.u.}$.

It is obvious that the curves of survival for the two treatments are the same when the electrostatic unit is used to measure the X-ray. The difference in the time curves is due to the fact that copper irradiation gives the same number of electrostatic units in less time than chromium. The straight lines of figures 4 and 5 are the theoretical curves plotted from the electrostatic unit equations.

The data on the ratios of females to males after irradiation are likewise presented in table 1. These data measure the survival value of the sex or

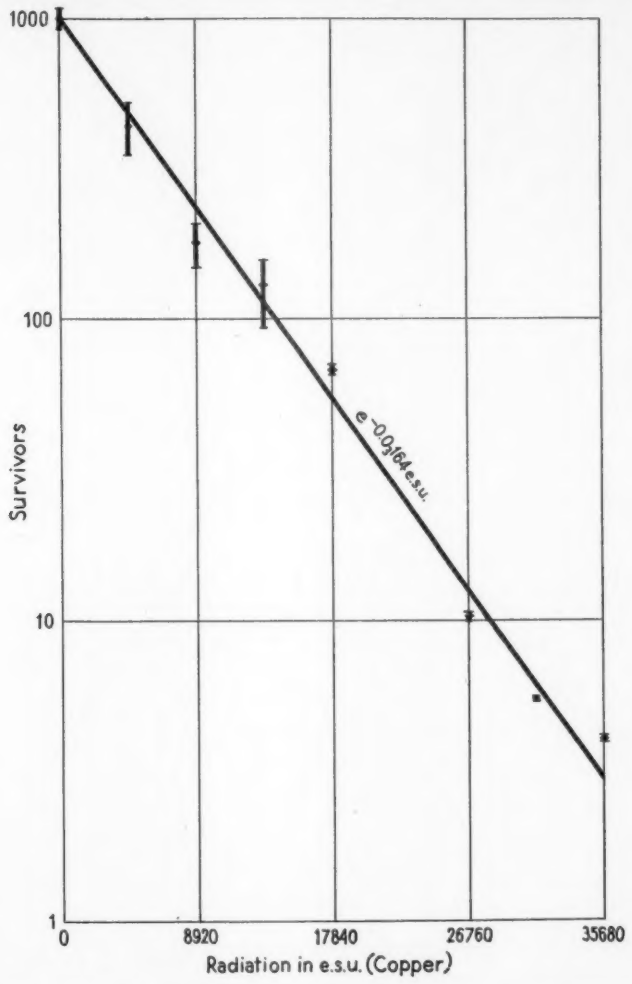

FIGURE 4.—Survival of Drosophila sperm irradiated with X-rays from a copper target.

X chromosome as separated from the other chromosomes, autosomes. Figures 6 and 7 present the curves for these two sex ratios plotted on semi-logarithmic scale. The cross bar shows the result of the particular treat-

ment and the heavy vertical line the probable error of this determination. There is more variation in these data than there was in those for the survival curve as the larger quantities of irradiation reduce the survivors

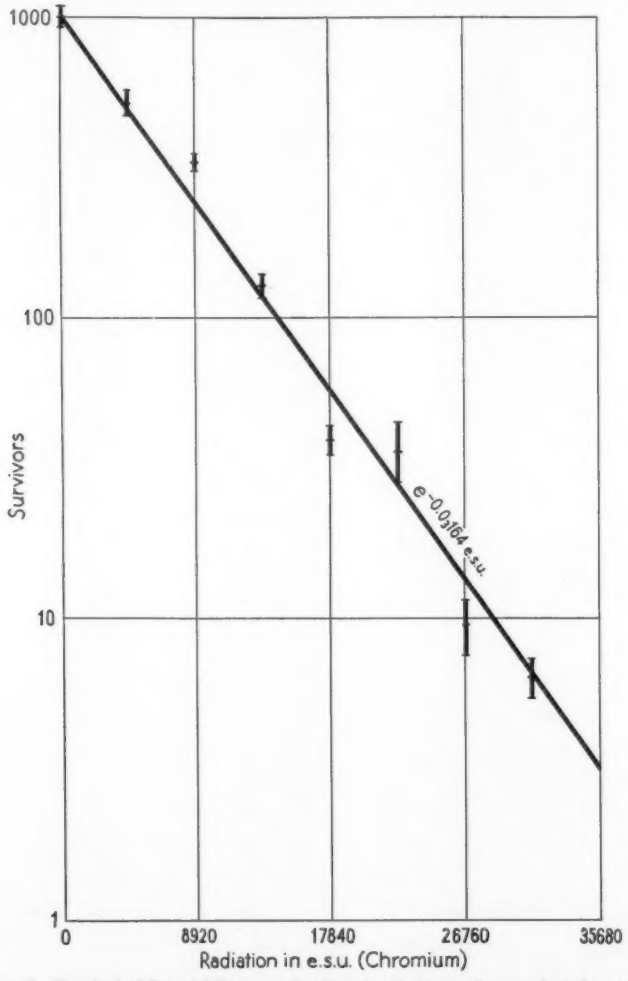

FIGURE 5.—Survival of Drosophila sperm irradiated with X-rays from a chromium target.

and consequently increase the observed variation. The data, however, clearly point to the linear nature of the semi-logarithmic plot. The equations for the changes of the sex ratios with increasing amounts of exposure

to the X-rays were for copper $Y = e^{-0.00114t}$ or $Y = e^{-0.0_577e.s.u.}$. The sex ratios of the flies surviving the chromium irradiation had the equations

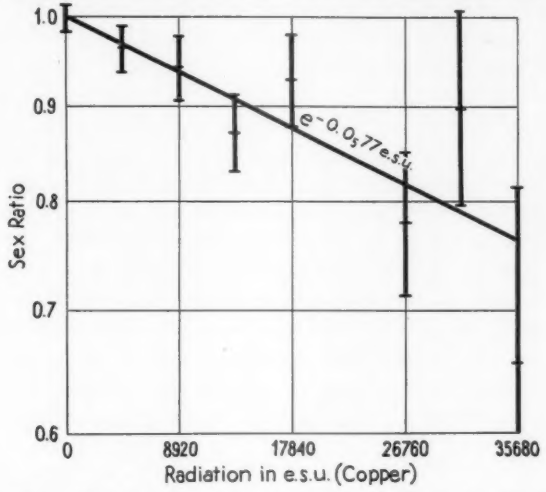

FIGURE 6.—Sex ratio of surviving Drosophila sperm after irradiation with X-rays from a copper target tube.

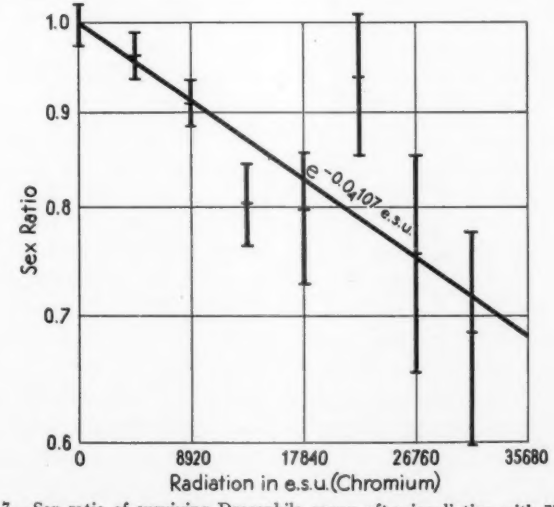

FIGURE 7.—Sex ratio of surviving Drosophila sperm after irradiation with X-rays from a chromium target tube.

$Y = e^{-0.00130t}$ or $Y = e^{-0.04107e.s.u.}$ The straight lines of figures 6 and 7 represent these equations.

It will be noted that the depression in sex ratio due to copper irradiation is different from that due to chromium. This difference is not significant since it has a standard error slightly more than itself ($P = 0.35$).

The recessive lethal mutations furnish data which cannot be collected from the dominant lethals, as this type of mutation does not kill the fly except when homozygous. This makes it possible to separate these lethals into pure lines, locate them within the chromatin matrix, etc. These mutations appear at random in relation to each other and to the visible mutations. If they appear at random as compared with the dominant lethals then the deaths brought about by the dominant lethals do not alter the form of the curve for the rate at which the recessive lethal mutations are produced. The assumption is borne out by the data.

Were it possible to count all of the recessive lethals produced, chromosomes in which only one lethal was produced, chromosomes with two lethals, three lethals, etc., then the form of the curve, representing percentage of lethals produced against electrostatic units of irradiation to which they were exposed, would be a curve of the form $Y = ant$.

There is a grave technical difficulty in determining the presence of double lethals in a chromosome when these lethals are close together. It is possible however to arrive at these same constants from the curve showing the percentage of chromosomes in which no lethal is produced. This curve is entirely like the survivor curve of the dominant lethals, the ant having the same meaning as in the curve above $Y = e^{-ant}$.

Table 2 gives the data for the percentage of sex-linked recessive lethals, including the doubles, as they were experimentally obtained. Beside this column is given the percentage of chromosomes tested which contained no recessive lethals. The percentages of visible sex-linked factors and dominant autosomal factors are given in columns 4, 5, and 6 for the copper irradiations and 10, 11, and 12 for the chromium irradiations.

The data of table 2 lead to the equations $Y = e^{-0.0_2165t}$ or $Y = e^{-0.0_4112e.s.u.}$ to describe the rate of recessive lethal production for sperm exposed to copper irradiation. The rates of lethal mutation for the sperm exposed to irradiation from chromium have the equations $Y = e^{-0.0_2162t}$ or $Y = e^{-0.0_4135e.s.u.}$ These data are plotted in figures 8 and 9.

A comparison of figures 8 and 9 shows that the rate of lethal mutation for sperm exposed to copper irradiation is somewhat less than that from chromium irradiation. The difference in the two rates is not significant, however, since the standard error of the difference of the two rates is 0.0_664 and the probability that the two distributions are alike is 0.15. The curves for the percentage of mutations with time of irradiation by X-rays showed that these gene changes are produced at a constant rate irrespective of wave lengths. If percentage of mutation is plotted against energy

TABLE 2

Relation between the X-ray dose and the ratio of recessive lethal or visible mutations.

TIME IN SECONDS	COPPER: SEX-LINKED MUTATIONS: RECESSIVE LETHALS	COPPER: SEX-LINKED MUTATIONS: CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS	COPPER: RECESSIVE VISIBLE	COPPER: AUTOSOMAL DOMINANTS: TESTED	COPPER: AUTOSOMAL DOMINANTS: UNTESTED
0	0.000±	1.000±	0.000	0.0000	0.0008
30	0.043±0.006	0.960±0.005	0.0054	0.0024	0.0062
60	0.089±0.013	0.911±0.013	0.0097	0.0008	0.0188
90	0.117±0.015	0.888±0.016	0.0152	0.0120	0.0142
120	0.200±0.021	0.813±0.021	0.0375	0.0066	0.0198
150	..	..	..	..	..
180	0.292±0.038	0.708±0.038	0.0152	0.0234	0.0132
210	0.350±0.051	0.675±0.050	..	0.0158	0.0158
240	No progeny	..	..	0.0303	..

TIME IN SECONDS	CHROMIUM: SEX-LINKED MUTATIONS: RECESSIVE LETHALS	CHROMIUM: SEX-LINKED MUTATIONS: CHROMOSOMES NOT CONTAINING RECESSIVE LETHALS	CHROMIUM: RECESSIVE VISIBLE	CHROMIUM: AUTOSOMAL DOMINANTS: TESTED	CHROMIUM: AUTOSOMAL DOMINANTS: UNTESTED	e.s.u.
0	0.006±0.004	0.994±0.003	0.000	0.0000	0.0030	0
37	0.068±0.010	0.935±0.010	0.0036	0.0025	0.0045	4460
72	0.124±0.011	0.878±0.011	0.0113	0.0048	0.0114	8920
111	0.193±0.017	0.815±0.017	0.0164	0.0106	0.0169	13380
148	0.265±0.036	0.779±0.034	0.0147	0.0130	0.0585	17840
185	0.188±0.029	0.825±0.029	..	0.0036	0.0216	22300
222	0.318±0.067	0.682±0.090	0.0454	0.0093	0.0278	26760
259	0.348±0.067	0.652±0.067	0.0435	0.0256	0.0342	31220
						35680

of the X-rays as the other variable, the curve shows that for a given quantity of energy the gene changes produced are constant. The wave lengths used in these experiments were rather long. HANSON and HEYS have used the gamma and beta rays of radium on Drosophila. In their experiments the rate of mutation, as measured by recessive lethals, increased directly with the dosage. In their first series of experiments we are unable to determine these dosages in electrostatic units. However, if we assign to one of their points a like value to ours in mutation rate, and dosage, we find that the two series of observations correspond. In another series of experiments they give a dosage of 6316 e.s.u. to their flies and obtain a mutation rate of 4.7 percent. This rate is somewhat lower than that obtained in our experiments. MULLER gives his tungsten X-ray irradiations for two points in e.s.u. units together with the rates of mutations. OLIVER gives like data which were taken in the same laboratory. For the e.s.u. readings these rates of mutation are considerably larger than those of either HANSON and HEYS or the writers. The fact that the radium

radiation produces essentially the same effects as the long waves of chromium and copper certainly suggests that wave lengths within these ranges are not important to the changes which X-rays produce in tissues.

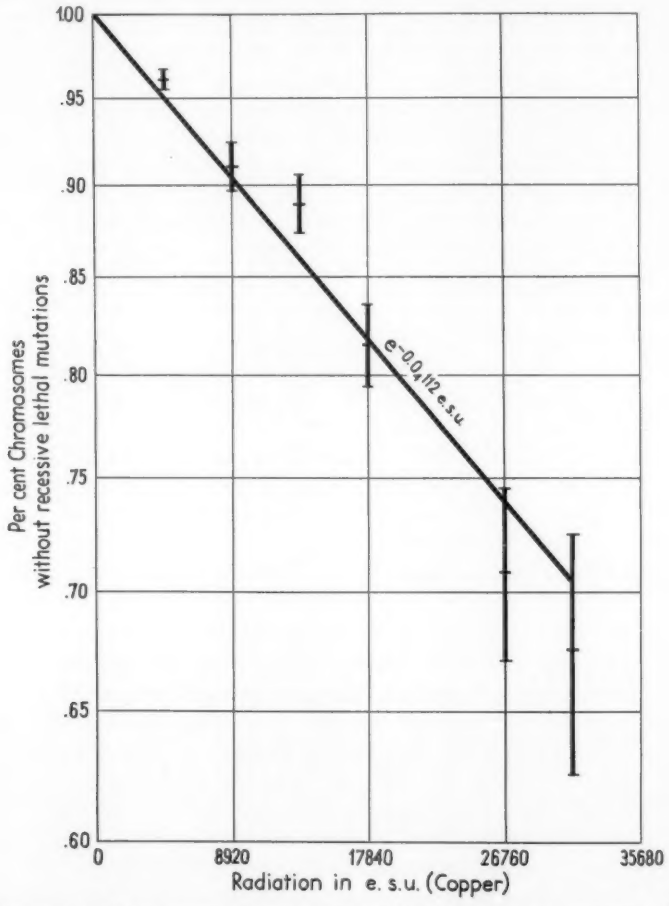

FIGURE 8.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from copper target.

It will be recognized that these diverse types of irradiation have produced their effects directly on the sperm without the intervention of crossing over. In this sense they would support STURTEVANT'S view that crossing over must be discounted as a probable explanation of so-called natural mutation.

The visible sex-linked recessive genes which were produced by the X-ray treatments cover a rather wide category of characters. The trends of the frequency of these mutations with increasing dosages of X-ray are

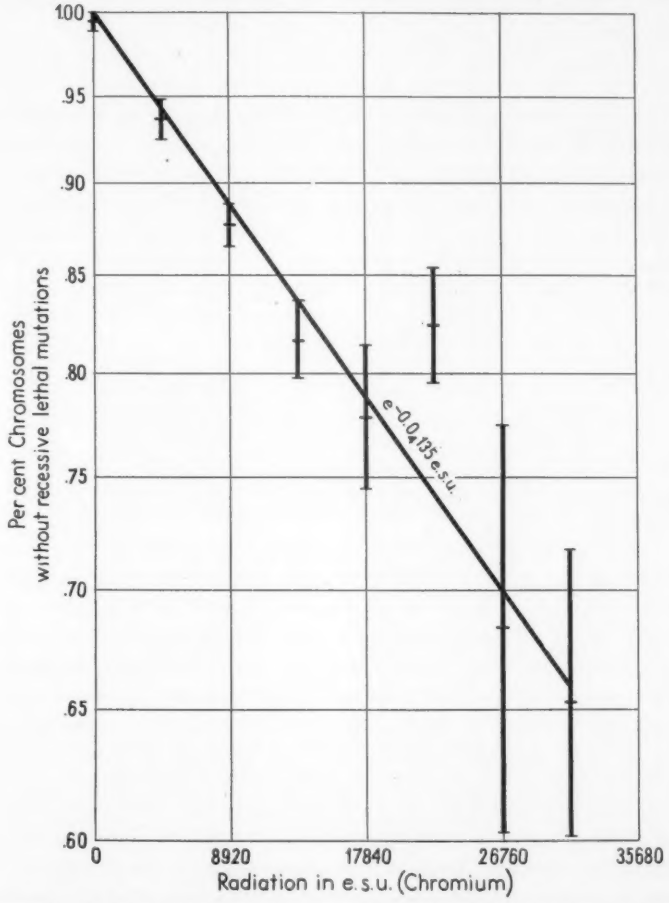

FIGURE 9.—Relation between the rate of recessive lethal mutation and exposure to X-ray irradiation from a chromium target.

given in table 2. This trend is quite irregular due to the relatively small numbers of these mutations produced. The rate of mutation rises with increase in the dosage of X-ray, however. A comparison of the rates of these mutations for the two types of X-ray leads to the conclusion that they are essentially the same. The number of visible mutations is about

one-tenth that of the recessive lethal mutations. In fact, for all data, there were 44 fairly clear and apparent visible mutations produced in the same group of sperm which produced 320 recessive sex-linked lethal mutations or 13.7 visible sex-linked mutations to 100 which were recessive sex-linked lethals.

The dominant, visible mutations in the autosomes, were divided into two groups. Each one of the first group was tested for location within its chromosome. Those of the second group failed to breed and were classified as untested. Here again it will be noted that these mutations increase with increasing X-ray dosage. The general trend of these curves is similar for both treatments; their irregularity is no doubt due to the small numbers of these mutations which were produced. The rate at which the dominant visible autosomal factors were produced is comparable with that of the recessive sex-linked visible factors. However, as the autosomes consist of about 10 times as much gene-containing chromatin as the sex chromosome, it is apparent that the dominant visible loci in the autosomes are more widely scattered than the loci for the visible sex-linked genes.

DISTRIBUTION OF THE GENE CHANGES

Three hundred and fifty-six mutated genes were tested for their loci within the sex chromosome. These loci were distributed over the sex chromosome's entire genetic length. The frequency distribution of the mutated genes was plotted on the conventional sex chromosome divided into units of 10, each series separately. The comparison of these distributions for the seven classes of chromosome length 0–10, 10–20, 20–30, 30–40, 40–50, 50–60, 60–70, showed a χ^2 of 10.1 for the six degrees of freedom. Because it is entirely probable ($P = 0.13$) that the two distributions are the same, they have been combined. Figure 10 shows these combined frequencies plotted as percent of the total mutations. The class interval for the first and second groups is 5 units of chromosome length. Each of the other intervals represents 10 units of the genetic chromosome.

Figure 10 shows much the same distribution of the mutated genes within the sex chromosome as found for the so-called natural mutation tabulated by MORGAN, BRIDGES and STURTEVANT, or for those produced by the heterogeneous irradiation of tungsten as tabulated by MULLER. (It is necessary to estimate the frequencies of MULLER's tungsten mutations from the graph, a method which, while approximately accurate, is not exact.) In common with these investigations there is an apparent close crowding of the mutation at the 0 end of the sex chromosome. This crowded area is followed by an area showing fewer gene loci. This depressed area is in turn followed by areas with mounting frequency of mutation to another crest at 50 to 60 units. From this point the number of the

genes per unit of genetic chromosome decrease to zero, at slightly beyond the 70 unit mark. One noticeable difference between our data and those cited is that no depression is noted in the region 40 to 50 units.

It seemed worth while to determine whether these three different groups of data could reasonably come from the same population. The comparison of the data of MORGAN, BRIDGES and STURTEVANT with this material showed a probability for their coming from the same population of 0.05. Comparison with the data of MULLER (1928) showed a similar probability, 0.02. The probabilities are on the borderline of significance. In the comparison over half the contribution to the χ^2 came in the first class, for on the basis of these other data, our relative frequency of factors in this

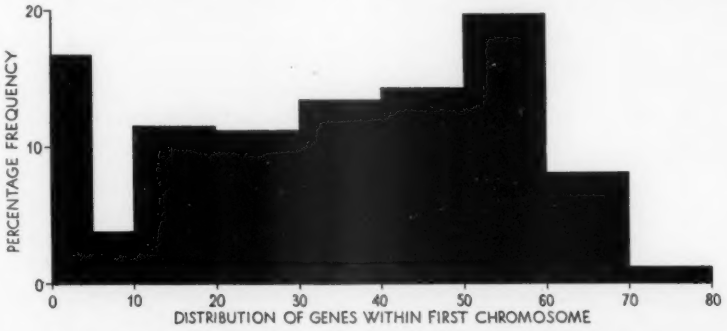

FIGURE 10.—Gene mutations as they were distributed within the genetic sex chromosome.

region is low. Another third of the contribution to χ^2 comes from the region 40–50 genetic units where we find a higher frequency of genes than do the other investigators. These differences may be significant. Since, however, the probabilities are in one case within three times the probable error and in the other but slightly beyond it, it seems likely that the differences in the three distributions are rather to be attributed to chance.

The mutant genes have been classified as lethals and visibles. These two classes intergrade and for that reason are more or less arbitrary. Figure 11 will enable the reader to appreciate the effects of these mutations on the vital processes of the organism. The data for the copper and chromium irradiations have been combined since comparison has shown them to be random samples of the same population.

Figure 11 shows that about 75 percent of this sample of genes are lethal. This means that the normal allelomorphs of 75 percent of this type of genes are vital to the animal's well-being. Seven percent show lesions which are severe enough to kill all but 1 to 5 percent of the progeny. Seven percent do not affect vital processes sufficiently to influence the organism's

viability to a marked degree. These results are comparable to those of MULLER on tungsten irradiation and of MULLER and ALTENBURG on natural mutations.

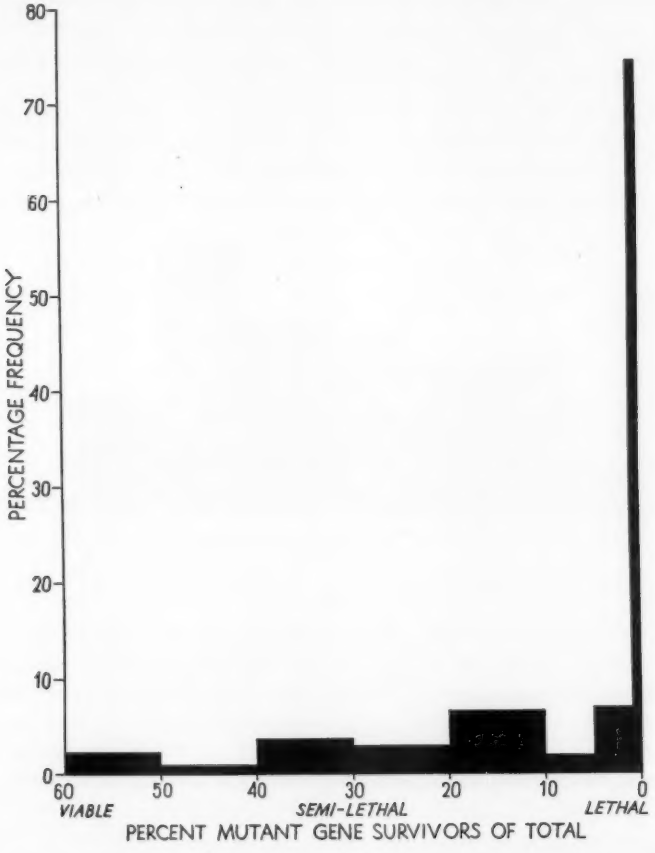

FIGURE 11.—Frequency distribution showing the sample of genes and the proportions of them which are completely lethal, produce pathogenic effects making them semi-lethal, and are fully viable. The ordinates represent percentage of genes in given class of the total. The abscissa gives the percentage of survivors in the gene mutant class divided by the whole number of survivors. 0=completely lethal; 50=normal viability.

Crossing over effects are noted with several of these factors. Aside from those which proved to be translocations between chromosomes, about 20 percent of the factors depressed the crossing over between w and B_x to about half its normal value.

The exact comparison of the visible genes one with another and with those found elsewhere is of particular significance to the argument which is to follow. For this purpose we shall consider only the factors which had fair viability, defined as 20 percent or better survival, classifying the rest as lethals. With this viability it seems reasonably certain that if any one of these factors had reappeared it would have been recognized. Our experiments produced 44 such factors, and 42 factors which, although generally lethal, occasionally produced a few living mutants. (These last 42 were classified as lethals, since they might have been completely lethal had they reappeared elsewhere. In all cases these 42 mutant forms, when they did survive, showed pronounced external abnormalities, no doubt but an indication of much more extreme changes within the internal organs.)

Of the 44 gene changes, which if duplicated should be discovered, 5 were proven to be allelomorphic to genes described by MORGAN, BRIDGES and STURTEVANT. These 5 loci were yellow (some differences from the previous yellow gene), echinus twice (the second echinus also showing differences), ruby once, miniature and forked. Besides these proven allelomorphic genes there were 6 others which were probably identical with others previously described. These genes were chlorotic, facet, singed (is ♂ sterile), ascutex twice (one more extreme than the other), furrowed twice (one more extreme than the other), and fused. The criteria for the conclusion that these genes were like those described are a similar morphology and location within the chromosome.

These data allow us to form an idea of the number of these genes within the sex chromosome. As a first approximation we shall assume that all of this class of genes are equally likely to mutate under the influence of X-rays and that when they do mutate they will be equally detectable. Two samplings have been made from this total population of loci. The first sampling followed the period of years during which the evidence for the existence of any of the loci came as the chance occurrence of natural mutations, thus giving rise to a Mendelizing difference. MORGAN, BRIDGES and STURTEVANT have tabulated and described these genes. The sex chromosome genes of this series, comparable to the 44 which we have described above, fall into 42 loci. If this same population of genes is sampled, the chance of obtaining a gene occupying any one of these loci in one draw is thus $42/\text{Total loci} \times 44$.

The experiment showed 6 proven identical loci and 8 very probable identical loci, or 14 in all. The experimental results set the answer to the above equation as 14; $42/\text{total loci} \times 44 = 14$ or $\text{total loci} = 132$. Thus the sex chromosome may contain 132 genes which can undergo rather easily detectable and fairly vital mutations. It may be argued that only the 6

proven allelomorphs should be used in the calculation. This would lead to an estimate of the number of loci as 308. We believe, however, that it is proper to consider 14 out of the 44 mutants as being allelomorphic to those previously known.

MULLER has published some data from which a similar estimate can be made. In 128 mutations of all kinds he obtained 20 which were at least 10 percent viable and conspicuous. Of these 20, 7 occupied loci previously known and tabulated among the 42 cited above; of these 7, 5 were proven allelomorphs. These data give 120 loci as the estimated number for this type of factors in the sex chromosome. Of the 7 loci found by MULLER in his tungsten X-ray work one, facet, was common to our series of 14.

The number of genes, 132, in the described category, must be regarded as a minimum, since if some of these genes mutate more readily than others we shall obtain a lower number than are actually present. Another independent estimate of the numbers of these sex-linked loci may be obtained from the ratio of the numbers of mutations noted once in the series to the numbers of those noted twice, three times, etc., after the manner developed by MULLER in his study of natural mutations. For our data 38 mutations appeared once or more; 6 appeared twice. If all of these loci are equally susceptible to the effects of the X-rays and if the genes within them change to produce equally detectable mutations then the ratio of the total number of these genes to the number that mutate once is equal to the ratio of the number that changes once to the number which changes twice, etc., or

$$\frac{N_{x+1}}{n_x} = r \text{ (a constant)}.$$

The series above gives the values of r as $6/38 = 0.158$. The value for the total number of the gene loci in this chromosome would consequently be 38 divided by 0.16 or 238, a somewhat larger figure than that obtained by the other method.

MULLER has applied this reasoning to the data published by MORGAN, BRIDGES and STURTEVANT for natural mutations. All chromosomes were used in this determination. A very real error is introduced here since examination of the data shows that sex-linked mutations are more easily detected at their original and repeated occurrences than are the autosomal factors. Furthermore calculations from these data relate essentially to the visible factors since any arrangement of this data would make it heavily overloaded with this class. Basing the calculations on the sex-linked visible factors, we find that 7 loci mutated 8 or more times; 8 loci 7 or more; 12 loci 6 or more; 14 loci 5 or more; 15 loci 4 or more; 18 loci 3 or more; 23 loci 2 or more and 42 loci 1 or more. This series leads to the values begin-

ning with the 8 appearing loci of 0.87—0.67—0.86—0.93—0.83—0.78 and —0.55. All values save the last are remarkably alike. The last value would seem to indicate either that genes under natural conditions have idiosyncrasies which make them characteristically high, intermediate, or low in their mutation rate, or that a mutation is more apt to be described once in this summary of genes than to appear in the list of repeated appearances, a purely spurious correlation. The latter might well happen in view of the tremendous difficulties involved in accumulating such data over a period of years. However that may be, the work of STADLER would argue for the first view. The consistency of the r values after that for genes appearing only once would argue against it. If there is heterogeneity in the rates between different genes then the number of genes, 76, obtained by using the $r=0.55$, will certainly be too low.

MULLER has estimated the gene number by another technique. From ZELENY'S and STURTEVANT'S data on the frequency of crossing over of the Bar gene with itself he reaches a value of 0.2 of a genetic unit as the space occupied by this gene. STURTEVANT'S paper of 1925 in conjunction with his later paper of 1929 would seem to make this rate too high. If we calculate the percentages of crossing over, assigning to the data double the rate of the more viable class where necessary, and weight the 14 different types of matings by the square root of their number we arrive at the figure 0.0009 as the percentage interchange in the askew crossing over, somewhat less than a tenth of a genetic unit for the Bar gene. This reasoning would lead roughly to a total of 800 loci for the genetic sex chromosome. But as MULLER points out, there are drawbacks in this evidence; the crossover relationships are disturbed by the presence of the Bar gene, as shown by the asymmetry of crossing over, and by the fact that the Bar region of the chromosome shows a larger than normal proportion of loci when plotted against the genetic chromosome.

This discussion leads to a numerical value for the loci of sex-linked recessive genes which produce visible, easily distinguishable, characters between 75 and 268, with an average of 189. Provisionally, we shall adopt the value 175 to represent these gene loci.

The question arises as to whether the lethal genes as a class have the same mutability as the visible genes. There are several facts which suggest that they have. The apparent similarity in the distributions of these two types of genes over the chromosome as regards time and space, points to the conclusion that no one gene or even a few genes can be so susceptible to change that they account for the observed results. The conclusion that the lethal genes have the same mutability as the visible genes is borne out by another type of evidence. The reasoning may be illustrated by the chromium data where the exposure was 17800 e.s.u. The chance of obtain-

ing a recessive lethal gene change in any one sex chromosome is the number of these mutations divided by the total number of chromosomes tested, or 18 divided by 68 = 0.265. If these changes are made at random, there should be chromosomes which show two recessive lethal mutations. Such chromosomes have been found. Our technique undoubtedly does not obtain them all, however, since the difficulty of separating and proving double lethal mutations increases markedly as their loci approach each other. We shall therefore consider only those cases in which one gene occupied a position in the chromosome left of miniature and the second between miniature and Beadex. The problem may be viewed as follows. There are 36 genetic units of chromosome left of miniature and 24 units between miniature and Beadex. In our experiment 26.5 percent of mutations were obtained in 100 chromosomes. If we assume that the genetic units between the factors, yellow and miniature, and miniature and Beadex represent the proportion of potential recessive lethal factors, the ratio of the loci becomes 36 and 24. In random sampling from a bag containing balls to represent these loci there would be 36 red balls representing the loci left of miniature and 24 white balls representing the loci between miniature and Beadex. Besides these there are 167 yellow balls, a number sufficient to represent the draws when no lethal is obtained. The chance of drawing a lethal is

$$\frac{36+24}{36+24+167}=\frac{60}{227}\text{or 26.5 percent}$$

as in the actual experiment. In any one draw the chance of obtaining a red ball is 36/227. In the second draw the chance of obtaining a second lethal in the mB_x region is the chance of drawing a white ball 24/227. The chance of drawing them both together is consequently $36/227 \times 24/227$.

But there is a second way in which this result may be accomplished since the white ball might be drawn first and the red one second. The chance of drawing a chromosome with two lethals each in the region indicated is $2(36/227 \times 24/227) = 0.0335$ or for the 68 draws of this experiment 2 chromosomes should be found with lethals in each of these regions. The experimental results confirm this hypothesis since we obtained 3 double lethals. Extending this process to the other irradiations the expectation of finding chromosomes with two lethals in these regions was, commencing with the initial irradiation, 4460 e.s.u., 0, 3, 3, 2, 1, 1, 1. The numbers actually obtained for the same arrangement were 1, 1, 2, 3, 1, 0, 0. The copper series is not quite in such good agreement, due partly we believe to the fact that it was the first experiment and that consequently our technique was not as good. The expectation for this series was 1, 1, 2, 3, 0, 3, 3. The observed results were 2, 0, 1, 2, 0, 0, 1. In both experiments fewer double lethals were found than expected, due partly we believe to our technique.

In spite of this difficulty the results are well within expectation since comparison of the observed with the expected leads to a P of 0.5. To this extent therefore these data support the view that recessive gene mutations are produced at random.

Testing lethal mutations directly for their rates of repeated appearance is an extremely time-consuming, tedious process, for the experiments must be performed with the autosomes. MORGAN, BRIDGES and STURTEVANT list cases of allelomorphism which have been found. The frequency of these cases is undoubtedly less than it should be when compared with the visibles which have been rediscovered, due to the technical difficulties just cited. The results so far are only useful in showing that recessive lethals do occasionally reappear.

The evidence seems to justify the assumption that the re-mutating value of the lethal factors is of the same order of magnitude as that for the visible genes. This assumption is utilized in the further discussion of the results which flow from this analysis of the experimental data.

DISCUSSION

The various lines of evidence may now be collected and examined in the light of the problem in hand,—the number, size and characteristics of the genes in Drosophila. Accepting the foregoing analysis as an essentially correct, if rough, description of the changes which take place in chromatin when Drosophila sperm are exposed to X-ray, and accepting the fact that there are approximately 175 visible, viable sex-linked genes, lead to the following numbers for the rest of the categories. The recessive sex-linked lethal genes are 7.3 times as numerous as the visible genes since in the experiment the ratio was 44 visible genes to 320 recessive sex-linked lethals; or there would be $175 \times 7.3 = 1280$ loci which could be occupied by recessive sex-linked lethals. The dominant visible mutations in the sex chromosome and autosomes appeared with a frequency comparable to that of recessive visible sex-linked factors, 175. The rate at which the sex-linked recessive mutations were produced was 0.0_4123 for each electrostatic unit. The rate at which the dominant lethal mutations in the sex chromosome were observed was 0.0_892 for each electrostatic unit. On a random chance distribution of these loci within this chromosome, the numbers of these two categories should be as their rates or 1.0:0.75. The probable number of loci which could be occupied by dominant lethals in the sex chromosome is $1280 \times 0.75 = 960$.

The relation of the numbers of loci in the sex chromosome to the numbers in the autosomes may be determined from the equations given earlier for the decline in the numbers which survive and from the decline in the sex ratio.

These data are gathered together in table 3. *Alpha* represents the number of quanta hitting the chromatin at a given time; *alpha* prime is the number of these quanta which are effective. As the data point to the conclusion that one quantum hit is sufficient to produce a lethal mutation, it follows that α'/α represents that proportion of the chromatin which is composed of these factors as related to the whole. The copper and chromium series are averaged for the volumes of chromatin occupied by the dominant lethal loci.

TABLE 3

	WHOLE SPERM CHROMATIN		SEX CHROMOSOME	
	COPPER	CHROMIUM	COPPER	CHROMIUM
Calculated α	1.163	1.405	0.199	0.240
Observed α'	0.024	0.020	0.0011	0.0013
Relative volume α'/α of vital gene chromatin	Average 0.0175		Average 0.00557	

Obviously the percentage of chromatin in the whole sperm capable of producing dominant lethal mutations is considerably greater than that found in the sex chromosome. This would seem to mean that the gene containing chromatin in the sex chromosome is less than that of the autosomes since the other possible hypothesis, that the average gene size in the sex chromosome differs from that in the autosomes, seems less likely. The ratio of these two values 0.00557/0.0175 would consequently be the amount of gene-containing material found in the sex chromosome as compared with the autosomes, or 32.4 percent.[7] This figure finds important confirmation in the work of STERN, PAINTER and DOBZHANSKY. By cytological study of the size of translocations involving chromosome fragments of known genetic length, PAINTER showed that the gene-bearing chromatin of the sex chromosome was only about 1/3 the length of the whole. STERN concludes that this same length is 1/2 the chromosome. DOBZHANSKY, on the other hand, making a similar study of second and third chromosomes, reached the conclusion that while the genes are unevenly scattered as in the sex chromosome they are found along the chromosome's entire length. These data consequently furnish a direct proof of these conclusions. The effective chromatin area of the sex chromosome is 9.8 percent of that of the autosomes as derived from the relation 5.27×10^{-9} cm$^2\times0.325\div17.51\times10^{-9}$ cm$^2=9.8$ percent. From this relation the number of autosomal loci carrying genes capable of mutating into dominant lethals would be 960/0.098 or 9800 loci. The loci which would contain genes capable of mutating to recessive lethals would be even greater than this, 13100 loci, since the number of

[7] If we calculate this percentage on the chromatin rather than the whole sperm this value is 26.5. Both percentages are subject to fairly large probable errors.

dominant loci is 0.75 that of the recessive loci. The number of loci which are capable of mutating to visible recessive factors of fair viability is, on the basis of sex chromosome proportions, 1800. These estimates of loci number are listed below to facilitate comparison.

TABLE 4

TYPE OF LOCI	NUMBER	PERCENT
Sex linked loci of visible factors	175	0.6
Sex linked loci of recessive lethal factors	1280	4.7
Sex linked loci of dominant lethal factors	960	3.5
Autosomal loci of visible recessive factors	1800	6.6
Autosomal loci of recessive lethal factors	13100	48.0
Autosomal loci of dominant lethal factors	9800	36.0
All chromosome loci of dominant visible factors	175	0.6

The numbers of these loci are considerably greater than we have been accustomed to visualize. The number 1975 for the visible recessive factors is similar to MULLER'S estimate except that in his estimate he considers this number as the whole number of factors,—due partly, no doubt, to the lack of information on lethals at that time. This list of factors does not include factor changes whose only expression may have been in the internal organs. This class of loci is likely to be rather small, however, since most physiological or other changes which have been noted have been accompanied by alterations in structure, which are visible externally on close examination.

The total number of loci within the Drosophila sperm should not be thought of as the sum of the different categories in table 4, for it seems likely that genes belonging to more than one category can occupy the same locus. We know this to be true in connection with dominant and recessive genes. It seems equally probable with regard to lethal genes since in allelomorphic series the viability of these genes is by no means the same, some being practically lethal while others are of nearly wild-type viability. The number of loci which could be regarded as the minimum is consequently 1280 for the sex chromosome and 13,100 for the autosomes. The proportion of the various categories gives the proportion of the different types of genes which may be expected to appear under the action of an agent capable of making the genes mutate.

This tabulation demonstrates that the animal's own genetic constitution is very significant from the standpoint of pathology. It suggests that there are not less than 14,380 loci occupied by genes which are vital to the normal morphology and well-being of the organism. A single factor may cause the destruction of the legs of the fly through development of joint lesions containing melanotic pigment, or it may make the fly pin-headed in ap-

pearance and inviable, etc. Yet these visible signs are but as the red flag of the auctioneer outside the shop announcing greater things going on within. Our study of the mechanism by which the animal's own genes prevent development of pathological conditions lags far behind the studies of pathogens and the lesions they make. Yet if all the external agencies which are known to produce such changes be tabulated they fall far below the possibilities of the organism's own genes in this direction.

The estimate of the number of genes and of the volume of chromatin occupied by these genes as furnished by the X-ray results make it possible to estimate the size of an average gene. Three independent estimates of the volume of the chromatin occupied by the different classes of genes are available. The first relates to the volume occupied by the loci containing genes capable of mutation to dominant lethals; the second to like loci of the sex chromosome; and the third to loci of the sex chromosome containing genes capable of mutating to recessive lethals. The numbers of genes of each class are all estimated from the same value, 175, for the sex-linked loci of the rather viable, visible, recessive genes. These estimates for the different volumes are shown below.

	SENSITIVE VOLUME	VOLUME OF CHROMATIN OCCUPIED	VOLUME OF LOCI
Dominant lethals (sperm)	0.0175	1.17×10^{-14}	1.1×10^{-18} cm^3
Dominant lethals (sex chromosome)	0.00557	1.00×10^{-15}	1.0×10^{-18} cm^3
Recessive lethals (sex chromosome)	0.00752	1.35×10^{-15}	1.0×10^{-18} cm^3

The sensitive volume is calculated from the methods and measurements given earlier, that is, by utilizing certain physical hypotheses. It is well to remember these hypotheses in interpreting the conclusions drawn. X-rays are assumed to be absorbed as units. An absorbed unit has a sphere of effect within which it acts. It is this purely physical quantity which is being measured. The result is biologically significant in that it sets the upper limit of the size of those elements whose alteration produces the observed results. The upper limit for the size of the gene is 1×10^{-18} cm^3. This value is below microscopic vision and therefore considerably less than the chromomeres assumed by BELLING to represent genes.

The possible configuration of the space relations may be obtained from the fact that the genes within the chromosome seem to follow one another as beads on the leptotene thread. The length of the sex chromosome which carries such genes is 0.50×10^{-4} cm. If we consider that every gene is capable of mutating to a recessive lethal, the total number of genes in this thread is 1280. At the leptotene stage this thread is much longer than it is in the condensed chromosome. The condensation could conceivably take place by either of two processes. The thread could contract to the length of the condensed chromosome or the thread could fold on itself in this manner

but keep its original length. If condensation by contraction took place the lineal dimension of a gene locus would be 0.50×10^{-4} cm $\div 1280$ or 3.9×19^{-8} cm. The measurement of the sensitive volume of 1×10^{-18} cm^3 then leads to the depth and breadth dimension of 5.0×10^{-6} cm.

The data on organic crystals show the interatomic distance to vary between 1A and 3A. The dimension of the gene locus 3.9×10^{-8} cm would allow but two atoms in the lineal dimension, a value which appears too low especially when the depth and breadth dimensions are so much larger. It seems preferable therefore to regard the condensed chromosome as composed of a folded thread of leptotene dimensions where the gene locus is rectangular or spherical. Such an assumption leads to a gene 1×10^{-6} cm on a side or there would be 50 atoms on a side of such a figure with 125,000 in all. The average protein molecule has a molecular weight of about 120,000. The average atomic weight of such a molecule approximates 15, or there will be about 8000 atoms in such a molecule. The measurements of the gene consequently make it possible that the gene is composed of something like 15 protein molecules. The results consequently make it probable that the gene size may be reduced beyond this limit. The use of ultra-violet light for the production of mutations may offer a means to this end since the critical volume in which it is absorbed is less than that of X-rays. The difficulty which lies here, however, is that of accurately measuring the ultra-violet absorption by the sperm.

SUMMARY

The significant points brought out in this paper may be summarized as follows. X-rays impinging on cell chromatin cause changes at multiple foci. These changes are heritable. They fall into 3 somewhat overlapping categories, dominant lethals, recessive lethals and viable mutations producing visible somatic effects. The killing effect of X-rays on the first cell generation comes from the production of dominant lethal mutations. The death of cells of the second generation comes from the recessive sex-linked lethals produced. The rates at which the changes are produced follow specific mathematical laws so closely that they may be used to measure the X-ray dosage which is given. The rate at which these changes are produced is dependent on the ionization value in air of the delivered irradiation. The characteristic wave length of the given X-rays seems to play no part in the effects produced.

From the standpoint of those interested in pathology, the most significant findings are those showing the genetic organization of this presumably rather simple animal. The number of separate inheritable loci within such an animal is in the neighborhood of 14,380. The proportions of the genes which occupied these loci were such that

48 percent were autosomal dominant vital factors,
36 percent were autosomal recessive vital factors,
6.6 percent were autosomal recessive factors producing visible effects,
4.7 percent were sex-linked dominant vital factors,
3.5 percent were sex-linked recessive vital factors,
0.6 percent were sex-linked recessive visible factors,
0.6 percent were sex-linked and autosomal dominant visible factors.

The importance of this comparison is the fact that within any animal there are a large number of genes which have the capacity for modification and when so modified produce definite visible pathologies or death. The protective and developmental mechanism which the animal has set up through its evolution is, thus, composed of many parts. This does not seem so strange when it is realized that each and every one of these 14,380 gene loci go to every cell in the body and that were all other tissues of the body removed the body could be seen as a shadow with its present form and structure in the genes which are left.

The proof that the chromatin is not all loci-containing is a significant fact as many workers believe that the presence of thymonucleic acid is diagnostic for such material. In this light, chromatin elimination as noted in tissue has often been misinterpreted as the direct throwing off of a portion of the inheritance. The results presented show that such need not be the case. They offer an explanation for the reported observations that in bacteria and yeast chromatin like that of the higher forms is absent.

The measurement of the size of the gene seems to be the first step in the study of the organization of these genes. The data are such that we can arrive only at a maximum size. This size is 1×10^{-18} cm.[3]

Consideration of the consequences which follow from regarding the condensed chromosome as a condensed leptotene thread or as a folded leptotene thread seems to favor the fold view. Adopting this hypothesis, the gene could be composed of as many as 15 protein molecules of average atomic size.

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THE MECHANISM OF MOSAIC FORMATION IN DROSOPHILA

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INTRODUCTION

The effects of X-radiation on the chromosomes make it possible to produce several different kinds of mosaics in *Drosophila melanogaster*. In general, these mosaics fall into two classes: (1) those that result from induced point mutations, and (2) those that result from breaks in the chromosomes. It is the object of this paper to describe the mechanism underlying the formation of mosaic flies that have resulted from breaks in the X chromosome.

The method for obtaining such mosaics has been described elsewhere (PATTERSON 1931). In brief, it consists in X-raying the wild-type fly and then crossing the treated individual to one carrying an X chromosome that contains several mutant genes scattered along its length. In case the X chromosome has not been affected by the treatment the heterozygous F_1 females will, of course, appear phenotypically normal. If, however, the irradiation has caused the loss, through breakage and elimination, of a piece of the treated X, the fact can be detected by the appearance of mosaic areas that show recessive characters in the F_1 fly. The particular recessive characters revealed will indicate the section of the treated X that has been eliminated, because the loss of a section containing the normal or dominant genes will allow such mutant genes as lie within the corresponding section of the untreated X to be phenotypically expressed.

Not all of the variant flies arising from zygotes that have received one broken and one unbroken X chromosome will exhibit mosaicism. The appearance of mosaic tissue depends upon the condition of the chromosome at the time the break was produced. If the chromosome is broken while it is in the single strand stage, the resulting fly will not exhibit mosaic areas, but will show the deficiency in all of its tissues, constituting what we have termed an aberrant fly. Such a variant may be either a male or a female, depending upon the region of the X chromosome that has been lost. The elimination of a piece from one of the strands of the two-strand stage of this chromosome is the condition necessary for the production of mosaic tissue, for in this event only a part (usually a half) of the zygote will inherit the broken or deficient chromosome. The resultant variant will be either a sex-mosaic (gynandromorph) or a mosaic female, again depending upon the particular region eliminated.

In previous experiments dealing with this subject it was found that owing to certain difficulties inherent in the stocks ordinarily used the data obtained could not be subjected to exact statistical treatment. The chief difficulty to which I allude lies in the fact that a zygote receiving an unbroken X and another X, from which a piece had been broken off of the left end by X-rays, usually did not survive. It was found that the few aberrant females which did appear in the F_1 cultures had lost but a very small piece from the extreme left end of their treated X chromosome. No female was obtained which had lost a piece by a break occurring as far to the right as the locus of prune. Since the X-radiation must break the chromosome at different levels, we should expect to find females containing chromosomes broken to the right of that locus. A study of this problem led to the discovery that the left end of the X chromosome contained a vital region, or a "gene for viability," which lies near the locus of broad. A female zygote will not develop unless this region is represented in duplicate (PATTERSON 1932a).

Attempts were made to obviate this difficulty by using as the treated parent a special stock called *Theta*. The X chromosome of the *Theta* fly carries a duplication in the form of a fragment attached to its right or fiber-bearing end. The fragment has the viability gene and the normal genes for yellow, scute, and broad, and consequently pieces of considerable length may be broken off of the left end of the *Theta* X without at the same time causing the death of a female zygote inheriting the broken element. While the *Theta* X has this great advantage over the wild-type X, yet it has one serious drawback. Aberrant females produced by breaks occurring at the left end, and falling within the range covered by the *Theta* fragment, cannot be detected in the F_1 cultures, because of the dominant effect of the wild-type genes contained in this fragment.

Since the simplest and presumably the most common type of break to be expected would be one causing the elimination of the left end of the chromosome, it is especially desirable to obtain as many as possible of the variant flies that could thus be produced. The problem was then to find an X chromosome in which the viability gene lay far removed from the left end. One might expect to find such a condition in a chromosome having an inverted section. Two X chromosomes known to have inversions were tested, but in each instance the viability region was found not to be included in the inverted section. At this point Doctor S. G. LEVIT called my attention to a stock, known as scute-8 apricot, which has in its X chromosome a long inversion. This inversion extends from a point lying just to the right of the locus of scute to some point lying between the locus of bobbed and the right end of the chromosome. The genes in the inverted section occur in reverse order, and hence the locus of bobbed lies near the left end

of the reconstituted chromosome, while that of the viability gene is situated well toward the right end.

In all of the experiments given in the following section of this paper, scute-8 apricot flies were treated and crossed to untreated yellow white crossveinless miniature forked-3 flies. The diagrams for these two chromosomes are shown in figure 1. It will be clear from the diagrams that if

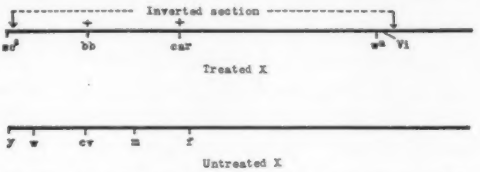

FIGURE 1.—The figure gives the diagrams of the treated and untreated X chromosomes used in the experiments. The upper diagram gives the composition of the scute-8 apricot chromosome, which contains a long inverted section. Note that the loci of white (w_a) and the viability gene are located toward the right or fiber-bearing end. The lower diagram shows the mutant genes carried by the untreated chromosome. These diagrams are based on the cytological map of the X chromosome as determined by the studies of MULLER and PAINTER (1932).

the left end of the scute-8 apricot chromosome is broken off and eliminated, the female zygote inheriting the untreated X and the broken X will show yellow body color, that is, the locus of yellow will be "deficient." Such a fly will be viable because the gene for viability will not have been eliminated by the break. She will also have heterozygous apricot eyes. A similar break occurring in one of the strands of the split chromosome will produce a mosaic female that will be half yellow and half gray.

EXPERIMENTS

Treated females

In the first experiment, young females, homozygous for scute-8 and apricot, were treated with a dosage of 1325 r units and immediately mated to males belonging to the recessive stock. For the first four days the flies were transferred to fresh bottles at the end of each twenty-four hour period, and after that they were changed at the end of each forty-eight hours, until the end of the tenth day. In this, as in all of the succeeding experiments, the cultures were kept in a room in which the temperature varied from 25 to 27.5 degrees Centigrade, with an average of 26 degrees. The flies for any lot began coming out on the eighth day and all had emerged by the end of the ninth day. The F_1 flies were examined for variants of all kinds, and a record kept of all individuals that had arisen from treated eggs fertilized by untreated X-bearing sperms.

The data from this experiment are given in table 1. They include for all, except the first daily period, the first five thousand flies counted for each

TABLE 1

Scute-8 apricot females, aged 0–12 hours, treated at 1325 r units, and crossed to yellow white cross-veinless miniature forked-3 males.

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		$y\ w^a$ FEMALES	$w\ c_p\ m\ f_3$ MALES	PARTIAL	HALF	w	w-f_a	f_a	m	f	
1	109	0	0	0	0	0	0	0	0	0	109
2	4,983	8	2	1	3	2	0	0	1	0	5,000
3	4,988	5	0	3	0	2	0	0	2	0	5,000
4	4,994	3	0	0	1	1	0	0	1	0	5,000
5+6	4,997	1	0	0	0	1	0	1	0	0	5,000
7+8	4,999	1	0	0	0	0	0	0	0	0	5,000
9+10	4,997	0	0	3	0	0	0	0	0	0	5,000
Totals	30,067	18	2	7	4	6	0	1	4	0	30,109

period. It was not practicable to attempt to obtain this number for the first period because of the fact that young females lay very few eggs within the first twenty-four hours. The variant flies, other than those due to point mutations, are listed under the general headings of aberrants, sex-mosaics, and lethal mutations. The point mutation cases are included with the non-variant apricot females. This method of recording reveals at once the frequency of occurrence of the different types of variants produced by deficiencies, for the total number of flies listed corresponds to the number of treated X chromosomes. Furthermore, the periodic transfer method shows the time in the germ-cell cycle at which the irradiation is most effective in breaking the chromosome.

Eighteen aberrant yellow apricot females were produced in a total of 30,109 treated X chromosomes, or about one in every 1672 chromosomes. It will be noted that the greatest number occurred on the second day (practically from the first eggs laid) and that they appeared in decreasing numbers for the succeeding days. Only two aberrant males were found, and both of these appeared on the second day. Among the eleven sex-mosaics, seven belonged to the partial type, that is, the fly was practically all female, with a small amount of male tissue present, such as a sex-comb, or male genitalia, or a small area of male coloration. Such flies usually do not result from X-raying, for they occur with about equal frequency among the controls. Most of them are due to the spontaneous elimination of one of the X chromosomes at one of the late somatic divisions. Four half-and-half gynandromorphs were found, three appearing on the second day, and one on the fourth day. The male parts of the first three had lost the maternal or treated X from one daughter cell at the first somatic division, while those of the fourth case had lost the paternal X. The three that had lost the treated X were probably caused by the X-raying. The flies were examined

for variations at the marked loci for white, facet (notched flies), miniature and forked, and all cases that showed, from breeding tests, the mutation to be lethal for the male (and therefore deficient) have been included in the table. Most of these came from eggs that had been laid during the first few days.

If we consider all of the variants to which the effect of X-radiation may be assigned, their frequency for the several periods of laying are as follows: one in 312 for the second day; one in 555 for the third day; one in 1000 for the fourth day; one in 1666 for the fifth period; one in 5000 for the sixth period; and none in 5000 for the last period. From this one may conclude that the nearer the female germ cell is to maturity at the time of treatment, the greater will be the effect of the irradiation in producing breaks and other disturbances in the chromosome.

The control flies for this experiment are given in table 2. The parent flies for the control series were taken from the same lots as those for the

TABLE 2

Scute-8 apricot females, aged 0–12 hours, and crossed to yellow white cross-veinless miniature forked-3 males.

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		$y\ w^a$ FEMALES	$w\ c_y\ m\ f_3$ MALES	PARTIAL	HALF	w	w-f_a	f_a	m	f	
1	502	0	0	0	0	0	0	0	0	0	502
2	4,997	0	0	2	1	0	0	0	0	0	5,000
3	4,998	1	0	1	0	0	0	0	0	0	5,000
4	4,997	0	0	3	0	0	0	0	0	0	5,000
5+6	4,999	0	0	1	0	0	0	0	0	0	5,000
7+8	4,999	0	0	1	0	0	0	0	0	0	5,000
9+10	4,999	0	0	1	0	0	0	0	0	0	5,000
Totals	30,491	1	0	9	1	0	0	0	0	0	30,502

experimental series, and were handled in exactly the same manner, except that the X-ray treatment was omitted. The only variant flies found were nine partial sex-mosaics, one half and half gynandromorph in which the male parts had lost the maternal X, and a single aberrant female.

The second experiment was similar to the first. In this, however, the females were "aged" for seven days before giving the treatment and mating to the recessive males. The counted F_1 flies are listed in table 3. The total number of flies obtained for the seven periods was 30,506 which is an increase of almost 400 over that secured in the first experiment. The increase is confined, of course, to the first day. The older females lay many eggs on this day, although the vast majority of them fail to develop. Under these conditions the cultures tend to become sour. In a measure, this difficulty can be overcome by "seeding" the food bottles with eggs laid for a

TABLE 3

Scute-8 apricot females, aged seven days, treated at 1325 r units, and crossed to yellow white crossveinless miniature forked-3 males.

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		$y\ w^{ch}$ FEMALES	$w\ c_v\ m\ f_3$ MALES	PARTIAL	HALF	w	w-f_3	f_3	m	f	
1	504	2	0	0	0	0	0	0	0	0	506
2	4,980	14	1	1	0	1	1	1	1	0	5,000
3	4,981	13	2	2	0	1	1	0	0	0	5,000
4	4,987	12	0	0	1	0	0	0	0	0	5,000
5+6	4,994	1	0	2	1	1	0	1	0	0	5,000
7+8	4,996	0	0	2	2	0	0	0	0	0	5,000
9+10	4,999	0	0	1	0	0	0	0	0	0	5,000
Totals	30,441	42	3	8	4	3	2	2	1	0	30,506

few hours by foreign flies, whose offspring can be easily recognized (for example, wild-type), before introducing the treated flies. The foreign larvae work the culture so that more individuals of the desired kind succeed in developing.

Among the F_1 flies of this series were found fifty-seven variants that could be attributed to the effects of X-rays. The frequency of their occurrence is one in every 250, 263, 294, 461, 1250, 2500, respectively, for the first six periods of laying, and one for the seventh or last period. The effect of aging virgin females, before treatment and mating, on the production of variants is clearly seen. As we have shown elsewhere, this is due to the fact that virgin females aged for several days retain most of their fully developed eggs in the ovaries, and, consequently, they have more eggs that are susceptible to the effects of radiation than is the case of younger or unaged females (PATTERSON, BREWSTER, and WINCHESTER 1932).

The control flies for the second experiment are given in table 4. Eleven

TABLE 4

Scute-8 apricot females, aged seven days, and crossed to yellow white crossveinless miniature forked-3 males.

DAYS	APRICOT FEMALES	ABERRANT FLIES		SEX-MOSAICS		LETHAL MUTATIONS					TOTALS
		$y\ w^{ch}$ FEMALES	$w\ c_v\ m\ f_3$ MALES	PARTIAL	HALF	w	w-f_3	f_3	m	f	
1	2,113	0	0	2	0	0	0	0	0	0	2,115
2	4,999	0	0	1	0	0	0	0	0	0	5,000
3	4,998	0	0	1	1	0	0	0	0	0	5,000
4	5,000	0	0	0	0	0	0	0	0	0	5,000
5+6	4,999	0	0	1	0	0	0	0	0	0	5,000
7+8	4,997	0	0	3	0	0	0	0	0	0	5,000
9+10	4,997	0	0	3	0	0	0	0	0	0	5,000
Totals	32,103	0	0	11	1	0	0	0	0	0	32,115

partial sex-mosaics, and one half-and-half gynandromorph were found. The male parts of the latter had lost the maternal X chromosome.

The interesting point brought out by these two experiments is the fact that no mosaic flies, other than the sex-mosaics, were produced by X-raying the female germ cells. The significance of this will be discussed in a later section.

Treated males

For the treatment of the male germ cells, two groups of unmated scute-8 apricot males were at first used. In one group the males were not over eighteen hours old, while in the other group they were five days old. By the time half of the projected experiments had been completed, it was seen that there was no fundamental difference in the results that had been obtained from the two groups. This was true both for the rate and for the character of the F_1 variant flies. It was then decided to combine the results under a single experiment. The tests were continued with males of various ages, but in no test were the males used more than five days old.

The males were given a dosage of 3975 r units, or exactly three times as strong as that administered to the females, and then mated to females of the recessive stock. This stock was balanced to the well-known ClB X chromosome. Both the homozygous females and the heterozygous bar females were mated to the males, but the F_1 bar females were of no use and were not counted or recorded.

Half of the males from any lot were treated, and half were not treated, the latter serving as controls. The periodic transfer method was employed, and for each culture twenty males and from thirty to forty females were placed in a food vial. At the end of each transfer period, the flies were etherized and the males and females separated. For the next period the males were again mated to virgin females, while the fertilized females were placed in a bottle containing food rich in yeast. At the end of six or seven days these females were removed from the bottle, and later their offspring in the bottle and its corresponding mating vial were examined and classified as in the previous experiments. It is possible roughly to tell, by this method, the age of the sperm (at the time of treatment) that fertilized a particular set of eggs, for all of the offspring of any given period came from eggs that were inseminated by sperm received by the females over a definite period of time.

In table 5 are given the data that were obtained in this series of experiments. The total number of flies that had come from eggs fertilized by treated X-bearing sperm was 20,303. In the last column are also given the number of flies obtained for each of the seven periods. The numbers vary from 244 for the sixth period to 6051 for the third period. This varia-

TABLE 5

Scute-8 apricot males, treated at 3975 r units, and crossed to y w c_v m f-3/C 1 B females.

DAYS	APRICOT FEMALES	ABERRANT FLIES		MOSAIC FLIES				LETHAL MUTATIONS					TOTALS
		y w^a FEMALES	w c_v m f_3 MALES	MOSAIC MALES	GRAY-YELLOW FEMALES	SEX-MOSAICS PARTIAL	SEX-MOSAICS HALF	w	w-f_a	f_a	m	f	
1	4,690	33	6	6	7	2	2	8	1	2	13	0	4,770
2	5,655	14	14	2	5	1	2	8	2	2	6	2	5,713
3	5,976	20	11	4	4	6	6	9	5	2	5	3	6,051
4	2,620	11	5	0	1	0	2	4	2	0	2	1	2,648
5+6	425	5	0	0	0	0	0	2	0	1	1	1	435
7+8	239	2	2	0	0	0	0	0	0	1	0	0	244
9+10	441	1	0	0	0	0	0	0	0	0	0	0	442
Totals	20,046	86	38	12	17	9	12	31	10	8	27	7	20,303

tion is due in part to the fact that the same number of cultures were not examined for each of the several periods, and in part to the fact that irradiation reduces the fertility, especially when applied to certain stages of the germ-cell cycle. There were forty-nine cultures carried through the entire seven periods, and a variable number for the first four periods. The best way in which to show the effects of irradiation on fertility is to calculate the average number of F_1 flies per culture. These calculations give the following results for the seven periods: first (114), 41.8; second (97) 58.8; third (105), 57.6; fourth (97), 27.3; fifth (49), 8.8; sixth (49), 4.9; seventh (49), 9.0. The figures in parentheses indicate the number of cultures examined.

In general, X-rayed males produce distinctly fewer offspring than untreated males. This is evidenced by the much larger number of offspring obtained from the corresponding control cultures. The average numbers of flies per control cultures for the seven periods were 285, 286, 251, 244, 271, 221, 208, respectively. In addition to this general reduction in fertility, there was also noted a differential effect for certain stages of the germ cells. The detection of the selective effect of X-rays was made possible by the periodic remating of the treated males to virgin females. As the figures given in the last paragraph show, the flies were less fertile on the first day than on either of the two succeeding days. This must be due to the effect of irradiation on the sperm cells that were the oldest at the time of treatment. On the fourth day there is a decided drop in fertility. In fact, the number of offspring produced is less than half of that for either the second or the third day. The three remaining periods show a still greater decrease, with the lowest average on the sixth period. The increase in the average for the seventh or last period above that for the sixth indicates, possibly, a slight recovery in fertility.

The extreme reduction in fertility, extending from the fourth day through the tenth day, must be due to the destructive effect of the irradiation on immature germ cells. The periodic remating of the treated males to a surplus of virgin females results in the rapid using up of the germ cells that were nearest to maturity at the time of treatment. The destruction by irradiation of the immature cells is certain to bring on a period of great infertility, or even that of sterility. This is in accord with the facts developed in the field of radiotherapy where it has long been known that immature cells are much more radiosensitive than are old cells (DESJARDINS 1932).

We may now consider the variant flies that were secured from the series of treated males, as shown in table 5. There were 246 variants (omitting the partial sex-mosaics, and correcting for controls) that could be attributed to the effects of X-rays. These occurred among 20,303 F_1 flies that had been derived from eggs fertilized by treated X-bearing sperms. This gives an average of one variant in every eighty-two flies. That is to say, one out of every eighty-two X chromosomes, treated with a dose of 3975 r units, resulted in producing some type of chromosome irregularity detectable among the F_1 offspring.

Of the eighty-six yellow apricot aberrant females, at least eighty-four (two were found among the controls, table 6) must have been the result of the irradiation breaking off the left end of the treated scute-8 apricot chromosome. This gives a rate of about one in every 240 flies. The rate of their appearance varies for the different days, being one in 144, 408, 302, 240, 87, 122, and 442 flies respectively, for the seven periods included in the table.

TABLE 6

Scute-8 apricot males, untreated, crossed to y w c_v m f-3/C 1 B females.

DAYS	APRICOT FEMALES	ABERRANT FLIES		MOSAIC FLIES			LETHAL MUTATIONS	TOTALS
		y w^a FEMALES	*w* c_v m f_3 MALES	GRAY-YELLOW FEMALES	SEX-MOSAICS PARTIAL	SEX-MOSAICS HALF		
1	5,132	1	0	0	0	0	0	5,133
2	6,007	0	0	0	1	0	0	6,008
3	6,049	0	0	0	1	0	0	6,050
4	3,170	0	0	0	1	0	0	3,171
5+6	1,497	1	0	0	2	0	0	1,500
7+8	1,500	0	0	0	0	0	0	1,500
9+10	1,499	0	0	0	1	0	0	1,500
Totals	24,854	2	0	0	6	0	0	24,862

There were exactly fifty aberrant males produced, at the rate of one in about every 406 flies. The aberrant male carries the untreated X and a treated X chromosome that has been deleted, that is, the middle section has been eliminated. It usually has gray body color, but shows the four

remaining recessive characters—white, crossveinless, miniature, and forked-3. However, twelve of these fifty males were mosaic for gray and yellow. The significance and a possible explanation of their mosaic condition will be discussed in the next section.

The next group of variants represents one of the classes with which this study is primarily concerned, namely, gray-yellow mosaic females. There were seventeen such females obtained, seven appearing on the first day, five on the second, four on the third, and one on the fourth. This class did not appear among the offspring from treated females (tables 1 and 3). These flies are characterized by having approximately one-half of the body gray and the other half yellow.

Twenty-one sex-mosaics were found, and of these nine were of the partial type, and the other twelve were typical half-and-half gynandromorphs. In each of these twelve flies the male parts had lost the treated X chromosome, or part thereof, at the first somatic division.

The last group of variants includes the so-called lethal mutation class. These represent deficiencies at one or more of the several marked loci indicated in the table. There were eighty-three such cases, of which thirty-one occurred at the locus of white, ten involving the loci of both white and facet (Notch), eight at facet, twenty-seven at miniature, and seven at forked. The writer has elsewhere discussed this type of variant fly (PATTERSON 1932c), but the point of interest here is that nine of these cases were "fractionals," that is, only a part of the fly showed the deficiency. For example, the fly would have one miniature wing and one normal wing. The number of these mosaics, occurring at the different loci, was as follows: four at white, one at facet, three at miniature, and one at forked.

The control flies for this experiment are given in table 6. The six partial sex-mosaics require no further consideration. The only other variant flies were two aberrant yellow-apricot females. Both of these had resulted from spontaneous breaks that had eliminated the left end of the scute-8 apricot chromosome. There were no lethal mutations found at any of the marked loci.

TYPES OF MOSAICS

Sex-mosaics or gynandromorphs

The experimental results presented above have brought out the fact that several different kinds of mosaic flies are produced, including the sex-mosaics. Seventy-two sex-mosaics were detected, and fifty of these belonged to what we have termed partial sex-mosaics. This type of fly is almost entirely female, but has a limited area of male tissue, such as a sex-comb, male genitalia, or male coloration on a part of the tip of the abdomen. In this series these mosaics occurred with equal frequency among the

control and experimental series. Among 80,918 flies of the experimental series, twenty-four partials were found. This gives a rate of one in every 3371 flies. Among 87,479 flies of the control series, twenty-six partials occurred, and this gives a rate of one in every 3364 flies. As stated above, these mosaics are usually not produced by X-rays, but are the result of spontaneous elimination of one of the X chromosomes in a relatively late somatic division. Occasionally, the elimination may occur at one of the early cleavage divisions, and if this should take place at the first division, there would be produced a half-and-half gynandromorph indistinguishable from one caused by irradiation.

In the treated female series, eight typical half-and-half gynandromorphs were found. The male parts of seven of these had lost the maternal or treated X chromosome, while those of one had lost the untreated or paternal X. One might infer that the latter case had occurred spontaneously, since the untreated X was missing, but, as I have elsewhere shown, there is an indirect effect of irradiation on the cytoplasm of treated eggs which not infrequently causes the elimination of the X chromosome introduced by the sperm (PATTERSON 1931). However, since two half-and-half gynandromorphs were found among the control flies, it is very probable that one or two of the cases found in the experimental series were also the result of spontaneous elimination.

In the treated male series twelve half-and-half gynandromorphs appeared, but none was found among the control series. The male parts of each of these flies had lost all or a part of the paternal or treated X chromosome. In five of the cases an extensive deletion had resulted in the elimination of the middle region of the X, so that the male parts were gray, but with the exception of yellow, they showed the other recessive markers of the untreated X. The male half of such gynandromorphs is the counterpart of the aberrant male, which is produced by a similar deletion that affects the single-strand stage of the chromosome. In the case of the gynandromorph, the deletion takes place in the two-strand stage, and affects only one of the two strands. Consequently, the initial somatic division gives the first opportunity for the deleted and non-deleted elements to separate into separate cells.

On this basis, one can readily understand why treating the female germ cell does not result in the formation of a gynandromorph that carries in its male tissue a broken or deleted X chromosome, because at the time of treatment the two-strand stage of the gametic chromosome has not been reached. X-raying the eggs, therefore, usually results in the production of aberrant males and females, although a gynandromorph is occasionally formed as a result of the elimination of one of the daughter X chromosomes at the first cleavage division. Gynandromorphs derived from X-rayed

mothers should not possess male parts carrying a broken or deleted X chromosome. I have, however, reported three such cases and attributed the breaks to the effects of X-radiation (PATTERSON 1931a). Two of these cases were due to the elimination of the X chromosome to which the *Theta* fragment was attached, but, since this phenomenon may occur spontaneously, these two cases do not constitute proof that the effect was due to X-rays. The third case represented one in which the X chromosome was broken at a point lying to the left of forked. It now seems probable that this case resulted from a spontaneous break which occurred after the beginning of maturation and before the first cleavage took place. This would give time enough for a break to take place after the split in the gametic element had occurred. This interpretation is strengthened by the recent discovery of a gynandromorph among untreated flies that had male parts with a broken maternal X chromosome.

At rare intervals gynandromorphs appear that cannot be explained on the simple elimination hypothesis, but can be accounted for on the basis of binucleated eggs. Such cases were first reported by MORGAN and BRIDGES (1919), and have since been discussed by L. V. MORGAN (1929). STERN and SEKIGUTI (1931) have described a mosaic male derived from a binucleated egg, but they were not able to determine whether the two nuclei were the partial products of the maturation divisions of an originally single egg nucleus, or whether the oocyte was binucleated from the first.

Mosaic females

Several different types of mosaic females are produced by breaks induced in one of the strands of the two-strand stage of the X chromosome of the sperm. One type is formed as a result of a single break, and is represented by the gray-yellow half-and-half mosaics. The fact that they are found in the treated male series, and not in the treated female series, at once suggests that they arise from sperm in which the gametic X chromosome is already split at the time of irradiation. Otherwise, it would be difficult to explain their mosaic condition. They appeared at the rate of one in every 683, 1142, 1513, and 2648 flies, respectively, for the first four days or periods (table 5). These numbers, however, are not large enough to show whether the difference in rate for the different days is significant. It is of interest to note that they appeared at a decreasing frequency for the successive days, but when all types of mosaics arising from split chromosomes are considered, there is little or no indication of a decreasing trend, the rate being one in every 530, 571, 503, and 882, respectively, for the first four periods.

The second type of mosaic female is found among the "lethal mutations" class of flies. These were found in the treated male series, and all appeared

within the first three periods. It is not always possible to determine by breeding tests on fractionals whether the variation is due to a point-mutation, or to a deletion, because cells carrying the affected chromosome may not have been included within the germ glands. Nevertheless, they are important in indicating the split condition of the X chromosome in the sperm at the time of treatment.

Eversporting types of mosaics

Another class of variant produced by X-rays includes some of the so-called eversporting mosaics. The most common type of this class is caused by a chromosome rearrangement involving the locus of white, so that flies heterozygous for red and white show mottled-eyes. Sometimes adjacent genes are involved, especially the locus of facet, resulting in the production of mottled-eyed and variable notched-wing flies. Many of these cases have not as yet received an adequate explanation, but the writer has found two cases that are due to unstable translocations. One of these has been worked out both cytologically and genetically. It was found that a piece of about six map units of the left end of the *Theta* X chromosome had been broken off and translocated to a fourth chromosome. This piece carried the normal genes for white and facet, and its occasional loss from the attached fourth during somatogenesis resulted in the production of mottled-eyes and variable notched wings (PATTERSON 1932b). Still another case belonging to this category was found several years ago, and was partly worked out before the stock was lost. In this stock the flies heterozygous for gray and yellow showed yellow spots scattered over the body. Linkage tests showed that a small piece containing the normal gene for yellow had been broken off and translocated to a third chromosome. The loss of this piece during development produced the yellow spots.

This same type of eversporting mosaicism may occur as a result of spontaneous elimination in untreated material. A very good example is found in the scute-8 apricot stock. If homozygous females are crossed to yellow white males, about one in every sixty-six F_1 females shows one or more yellow spots on the body (4952 examined). Irradiation does not appreciably increase this rate, for in the treated series, one in every fifty-seven F_1 females had yellow spots (2165 examined). That the spotting is not due to a mutable gene is indicated by the fact that the scute-8 F_1 males are never spotted.

This case may be brought into line with the three cited above if we assume that the left end of the scute-8 X is occasionally lost during the course of development. This interpretation is made probable by a few tests that have been made with F_1 females. Three non-spotted and five spotted females were backcrossed to yellow white males. The three non-spotted

and four of the spotted females gave no spotted offspring. The fifth spotted female gave eighteen F_2 females, all showing yellow spots, but failed to yield any scute-8 apricot males. The stock established from this line continues to breed in this same manner. The loss of the left end of the scute-8 X creates a deficiency and hence, since it occurs in every X, the scute-8 apricot male zygote is non-viable. That the break is not followed by the translocation of the piece to one of the autosomes is made certain by the fact that none of the white-eyed females in the cultures are ever gray.

Mosaic males

We have already mentioned the fact that mosaic males are found in the treated series. These flies display various degrees of coarse and fine mosaicism, from types showing half the body gray and half yellow, with a sharp line of demarcation between the two halves, to types showing a fine mosaic of small gray and yellow areas (pepper and salt types). These flies arise as aberrant males and are therefore hyperploid. They have the untreated X, marked with the five recessive genes, and a deleted fragment of the treated X chromosome. The mosaic condition applies only to the yellow locus, for the other four recessives always show. The mosaic can be explained on the same basis offered above to account for some of the eversporting types of females, that is, the left or gray end of the deleted X is sometimes lost during the cleavage stages. If the elimination occurs once and at the first division, a half-and-half mosaic male would result, but if it took place at a later division, a male with a yellow spot would be produced. The fine mosaic type would result from several eliminations occurring in the late cleavage stages. It is possible that some of the mosaic males, as well as some of the aberrant males, have arisen from treated gametes in which the gray end had been translocated to another chromosome, rather than by a reattachment with the right-hand end of the deleted element. This cannot be determined genetically because of the fact that such males have no Y chromosome and are consequently sterile.

There are other types of mosaics than those obtained in this series of experiments. Thus by X-raying larval stages, heterozygous for various mutant characters, it is possible to produce "somatic mutations" that appear in the adult fly as mosaic or variant areas (PATTERSON 1929a, 1929b). Some of these areas are the result of induced point mutations, but most of them have been brought about through the elimination of a section of the chromosome that carried the dominant genes. The writer has also reported the finding of mosaics that resulted from somatic segregation of chromosomes, caused by X-raying egg and larval stages, heterozygous for yellow and singed (PATTERSON 1929c).

Mosaic flies caused by the loss of autosomal material are also known to oc-

cur. BRIDGES (1921) showed that the small fourth may be lost through nondisjunction. This gives rise to haplo-IV flies that have fine delicate bristles, a condition known as "Diminished." Recently MOHR (1932) has described and tested a fly that was normal on one side and haplo-IV on the other. DOBZHANSKY (1932) has reported a case (his translocation H) in which a small piece, including the locus of purple, had been broken out of a II chromosome and translocated to a Y chromosome. He obtained two females that were deficient at the locus of purple, showing that such hypoploid females may be viable. Finally, STERN (1927) has described two cases of mosaic spotting, caused by spontaneous eliminations of parts of the III chromosome. In one case the tissues of the mosaic spot were deficient for the left arm of the third, and in the other for half of the right arm of this same chromosome.

In general, however, the loss of either one of the two large autosomes, or even of more than a small piece thereof, is fatal. With respect therefore to the viability of the fly, the elimination of autosomal material stands out in sharp contrast to the loss of X-chromosomal material. This fundamental difference may have been the outcome of a long selective process. Through the evolution of the sex-determining mechanism, the fly has become adapted to the absence of one of the X chromosomes (but not to the absence of a large autosome) and consequently one can bring about the elimination of a very considerable portion of one of these chromosomes in an XX zygote without causing the death of the individual.

DISCUSSION AND CONCLUSIONS

In the majority of cases, the fundamental cause underlying the mechanism of mosaic formation is the process of chromatin elimination. Another important fact concerning mosaic formation relates to the condition of the chromosome at the time the irradiation is applied. The general rule is that if the chromosome is not split, a break or deletion will produce an aberrant male or female, depending upon the particular region eliminated; but if the chromosome has already split and is in the two-strand stage, a break or deletion in one of these strands will give rise to a mosaic fly. To a certain extent, one can obtain a desired type of variant by applying the irradiation to certain stages of the germ cells or larvae.

The results obtained in this and previously reported experiments indicate that, with few exceptions, breaks and deletions induced in the egg chromosome by X-raying virgin females give rise to aberrant flies only. We should expect this to be the case from the work of HUETTNER (1924) on maturation and fertilization. He found that the polar bodies are not given off until after the sperm has entered the egg. The ovarian egg has a large, reticular nucleus, but in eggs about ready to be fertilized the meta-

phase spindle of the first polar body is present. In treating the egg in the ovary, the irradiation is applied to chromosomes that in the main are in the tetrad or pretetrad condition, and, consequently, a deficiency induced in any chromosome will affect both of its potential strands. A mature egg that retains the broken element will, upon fertilization, develop into a fly that carries the deficiency in all of its cells, that is, it will be of the aberrant type. If mosaics with deficient chromosomes are produced at all, the break must have taken place in those eggs that had formed the first polar spindle, where there might be a chance for the gametic split to occur.

The production of mosaics from treated eggs is confined mainly to sex-mosaics of the half-and-half type. Such mosaics or gynandromorphs do not carry a broken chromosome (one exception, explained above), but are the result of the elimination of an entire somatic chromosome, usually at the first cleavage division, and therefore following maturation and after the gametic split had occurred. It has been found that the rate at which half-and-half gynandromorphs appear among F_1 flies derived from treated females is increased about three times over that at which they are found among the controls. It has also been observed (MORGAN and BRIDGES 1919, PATTERSON 1931) that the maternal and paternal X chromosomes are eliminated with equal frequency in gynandromorphs derived from untreated flies. In the case of gynandromorphs from treated mothers, the maternal and paternal chromosomes are likewise lost with about equal frequency.

This fact lead the writer (1931, p. 199) to suggest that at least part of the effect of irradiation on elimination must be indirect, probably operating through the cytoplasm of the egg. If this suggestion is valid, then the cytoplasm must eventually recover from this effect. In the present series of experiments little or no evidence was found which indicated that treating the egg increased the type of sex-mosaic which had male areas of less than half the size of the body. This type of gynandromorph occurred as frequently among the control flies as among the experimental flies. However, in some of the previously conducted experiments, especially when the *Theta* stock was used as the treated parent, evidence was found indicating that the effect persisted beyond the first cleavage division, for here the partial sex-mosaics were significantly increased as a result of the treatment.

If the cause of the elimination is brought about through the absorption of the rays by the cytoplasm, there might be a recovery from the effect causing elimination as development progressed. Recovery from the effects of radiation by organisms has been reported in the literature. Thus BOVIE and KLEIN (1918), and BOVIE and DALAND (1923) showed that paramecia, sensitized to heat by exposure to fluorite radiation, recover from the effects of the radiation after about five hours, and HANCE (1926) found that X-

radiation of these same organisms brings about a slight but constant depression in the division rate from which the organisms recover after from two to five days.

Irradiating the sperm cells by treating adult males results in the production, not only of aberrant flies, but also of mosaics, including gynandromorphs. But the increase in gynandromorphs over that found in controls is restricted to the half-and-half type. The partial sex-mosaics show no appreciable increase as a result of the irradiation. I have obtained a total of one hundred and nineteen half-and-half gynandromorphs among flies derived from treated males. Their distribution with reference to the elimination of the treated or untreated X chromosome is entirely different from that reported above for the treated female series. One hundred and thirteen of these had lost the treated X (or part) from their male parts, and only six had lost the untreated or maternal X. Undoubtedly, some half dozen of those in which the treated X was found to be missing were not caused by the irradiation but were the result of spontaneous eliminations. This leaves one hundred and seven cases that can safely be attributed to the direct effects of irradiation. In forty-five flies the male parts carried, in addition to the marked maternal X, a broken or deleted paternal X. In all such cases the irradiation must have affected only one of the two gametic strands. The remaining sixty-two cases gave the appearance of having lost one entire gametic strand of the treated X chromosome. This was proved to be true in some cases by the use of the *Theta* stock in which the gray fragment at the right end of the X chromosome served as a marker. In cases derived from treated non-*Theta* stock, it is possible that in some instances the treated X had been broken at a point lying to the right of the normal allelomorph of the last marker and the right-hand end of the chromosome, for in this event the presence of the broken chromosome could not be detected.

The mosaic females derived from treated sperm fall into two classes, the gray-yellow class and the fractional or lethal class. Both of these classes are important, because both indicate that the breaks which caused them took place in only one of the strands of the two-strand stage. This raises the question as to why X-rayed sperm give rise to both aberrant and mosaic flies. The ratio of aberrant females to mosaic females is about six to one. With reference to the condition of the gametic chromosome at the time of treatment, there are three possibilities, as follows: (a) The chromosome may not be split in any of the sperm, (b) it may be split in all sperm and (c) it may be split in some sperm but not in others. We may now consider what would follow on the basis of each of these assumptions.

If we assume that the chromosome is not split in any of the male gametes at the time of treatment, the appearance of aberrant flies can easily be

accounted for, but in order to explain the appearance of mosaic females, it would be necessary further to postulate some kind of delayed radiation effect which would bring about the break in one of the strands after the split had occurred. Aside from the fact that we have been unable to obtain any experimental proof of a delayed effect in breakage, there is the further objection that no such effect occurs in the egg chromosome, which is known to be in the one-strand stage at the time of treatment.

The second suggested possibility that the gametic split is present in all fully formed sperm also meets with a serious objection. It is true that some cytologists hold the view that the gametic split occurs in the prophase stages of the preceding division; nevertheless, the genetic evidence is not in harmony with that assumption. On the basis of this suggestion it would be necessary to assume that radiation sometimes breaks one strand and sometimes both strands. If the break is the result of a "direct electron hit," it would be difficult to explain the fact that in about six times out of seven both strands are hit simultaneously.

The simplest explanation to account for the facts of breakage is implied in the third possibility, for the genetic evidence indicates that the gametic split is present in only about one out of every seven fully formed sperms. Our complete lack of knowledge with reference to the exact nature of the effect of radiation in producing breaks in the chromosomes makes it difficult to decide definitely between these three alternatives. In view of all of the facts, however, the writer regards the third alternative as the most probable.

Another point of interest relates to the fate of the broken-off or eliminated piece of the chromosome. In all probability this piece simply disintegrates in the cytoplasm, although the possibility of its loss through the mechanism of a translocation must be considered. Obviously, such aberrant flies as the yellow apricot females, derived from treated male gametes, could not have arisen through a translocation of a part of the broken scute-8 chromosome to one of the autosomes. The irradiation must produce such translocations, but these can not be detected by examination of the F_1 flies, for the deficiency created at the locus of yellow in the broken X would still be covered by its normal allelomorph in the translocated fragment on the autosome.

The corresponding yellow apricot females, derived from treated female germ cells, might possibly arise from translocations. For here the break is induced in prematuration chromosomes and should a translocation occur there would be an equal chance that the resulting gametes would fail to receive the translocated piece. Such gametes upon fertilization by yellow X-bearing sperms would produce yellow aberrant females. But if this were the method, then there would be an equal number of gray hyperploid

females produced. It should be possible to detect at least some of these postulated females without having to resort to breeding tests. In over sixty thousand F_1 gray females from cultures that yielded sixty yellow aberrant females, not a single hyperploid was detected, although the flies were carefully examined with the view of detecting such variants. In view of these facts, it is very probably that the yellow aberrant females from treated mothers have been produced by a simple break, followed by the disintegration of the broken-off piece.

The class of gray-yellow mosaic females might possibly come from induced translocations. These flies are derived from treated sperm, and if the broken-off fragment from one of the strands were to become attached to an autosomal strand, it might segregate into the future gray part of the fly at the first somatic division. If this should happen, some of the sons of those mosaic females yielding gray offspring would reveal the presence of the translocated piece. Seven of the tested mosaic females gave numbers of gray offspring large enough to make the test adequate, but in no case were males carrying a translocated piece obtained. Such a male would have gray body color, but would show the remaining recessive characters present in the marked maternal X chromosome. This evidence supports the conclusions that the gray-yellow mosaic females result from simple breaks in the treated X chromosome.

SUMMARY

There is reported in the paper a series of new experiments which had as their object a more exact determination of the nature of the effects of X-radiation on the production of mosaic flies by breaks in the X chromosome. The scute-8 apricot stock used in the experiment is characterized by having an X chromosome with a long inverted section. This condition made it possible to obtain considerable numbers of aberrant and mosaic flies by breaking off the left end of the X chromosome—a result that is rarely attained when a normal X chromosome is treated. The main points brought out in the experiments are summarized in the following paragraphs.

1. The main cause underlying the production of mosaic flies is chromatin elimination. The elimination may involve the entire X chromosome but frequently includes only a part of that chromosome.

2. The type of fly resulting from breakage depends upon the condition of the chromosome at the time the break is induced. If the gametic element is not split at this time, the resulting fly will be of the aberrant type, and will reveal the deficiency in all of its tissues; but if the gametic split is present, the elimination of all or part of one of the strands will produce a mosaic individual.

3. It was found that if female germ cells were treated by X-raying vir-

gins, only the aberrant type of fly developed, following breakage of the X chromosome. This was taken to mean that the gametic split had not developed at the time of raying. The number of sex mosaics found among F_1 flies from treated mothers is significantly increased over that occurring among the controls. There is no conclusive evidence that these sex-mosaics carry a broken X chromosome, but they do show that their male parts lose the paternal X with the same frequency as the maternal X. This lead to the suggestion that the effect of radiation on the elimination of the X chromosome is in part indirect, probably operating through the cytoplasm.

4. X-raying mature sperm cells result in the production of both mosaic and aberrant flies, in the ratio of one to six. This indicates that at the time of treatment the X chromosome is split in about one out of every seven sperms. Treating the male germ cells also increases the number of gynandromorphs. Over forty percent of these carry a broken X chromosome in their male parts, but most, if not all, of the remaining sex-mosaics have lost half the treated paternal X at the first somatic division. There is no evidence that the treated sperm cell affects the elimination of the maternal X chromosome brought into the zygote through the egg.

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SEGMENTAL INTERCHANGE IN CHROMOSOMES OF TRADESCANTIA

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Chromosome ring formation has been found in a number of different genera. In certain species of Oenothera and of Hypericum and in the monotypic genus Rhoeo, all the chromosomes may be united in a single ring or chain at meiosis. Ring formation is maintained in these plants by a system of balanced lethals. In Datura, Zea, Pisum and Campanula rings of four or more chromosomes have been described. In most species with either large or small rings there is considerable pollen sterility due to non-disjunction at meiosis.

BELLING's (1927) hypothesis that ring formation is caused by segmental interchange between non-homologous chromosomes has been confirmed by the cytological and genetic evidence in Oenothera hybrids (CLELAND and BLAKESLEE 1931, EMERSON and STURTEVANT 1931) and in Zea (MCCLINTOCK 1930, BURNHAM 1930, 1932, BRINK and COOPER 1931, 1932) and from a study of chromosome arrangement in Rhoeo (SAX 1931). The behavior of reciprocal translocations in Drosophila (STURTEVANT and DOBZHANSKY 1930) is also in accord with BELLING's hypothesis.

The different types of chromosome configurations in the rings or chains is of interest in connection with the mechanism of chromosome segregation at meiosis. Interlocking of bivalents and rings has been found in several ring-forming genera, and the relations of these locked pairs of bivalents have an important bearing on the nature of chiasma formation. Little is known concerning the mechanism involved in producing segmental interchange. Chromosome ring formation in Tradescantia provides an opportunity for a more complete study of these problems.

CHROMOSOME PAIRING IN NORMAL AND SEGMENTAL INTERCHANGE TRADESCANTIAS

Chromosome pairing at meiosis has been studied in normal diploid plants of *Tradescantia bracteata* Small, T. *edwardsiana* Tharp, *T. reflexa* Raf., and *T. gigantea* Rose; in a tetraploid *T. occidentalis* Britton; in several segmental interchange plants of *T. edwardsiana* and *T. reflexa;* and in *Rhoeo discolor* Hance. The analysis of chromosome configurations is based on aceto-carmine smears. In most cases, a small proportion of aceto-haemotoxylin was added to the aceto-carmine. After the smears were covered, the slides were heated gently and then inverted and slightly

The accompanying Heliotype plate is paid for by the GALTON AND MENDEL MEMORIAL FUND.

Explanation of Plate 1

Photomicrographs of Tradescantia chromosomes from aceto-carmine smears. Magnification ×1500, except figure 11 which is ×1000. The outline of each photograph corresponds to the cell wall. Figures 1 to 9 inclusive are from preparations of *T. edwardsiana* with segmental interchange chromosomes.

Figure 1.—Chain of four chromosomes with terminal chiasmata and four bivalents. The orientation of adjacent chromosomes towards opposite poles in the chain will result in regular disjunction and fertile pollen grains. The bivalent below the chain is a double ring with three chiasmata.

Figure 2.—Regular disjunction type of chain of four chromosomes with two subterminal chiasmata. The middle chiasma in the chain is almost always terminal.

Figure 3.—The chain of four chromosomes is interlocked with a ring bivalent. This chain is also of the disjunctional type and has one subterminal and two terminal chiasmata.

Figure 4.—A non-disjunctional type of chain with one subterminal chiasma. The typical bivalent configurations—3 rings and 1 rod—are clearly shown.

Figure 5.—A non-disjunctional chain with one subterminal chiasma. Two rod bivalents are shown,—one with a terminal and the other with a subterminal chiasma.

Figure 6.—A non-disjunctional chain with two subterminal chiasmata. This characteristic position of the subterminal chiasmata indicates the relative lengths of the segments in the interchange complex.

Figure 7.—A ring of four chromosomes with two ring and two rod bivalents. Rings of four chromosomes were found much less frequently than chains.

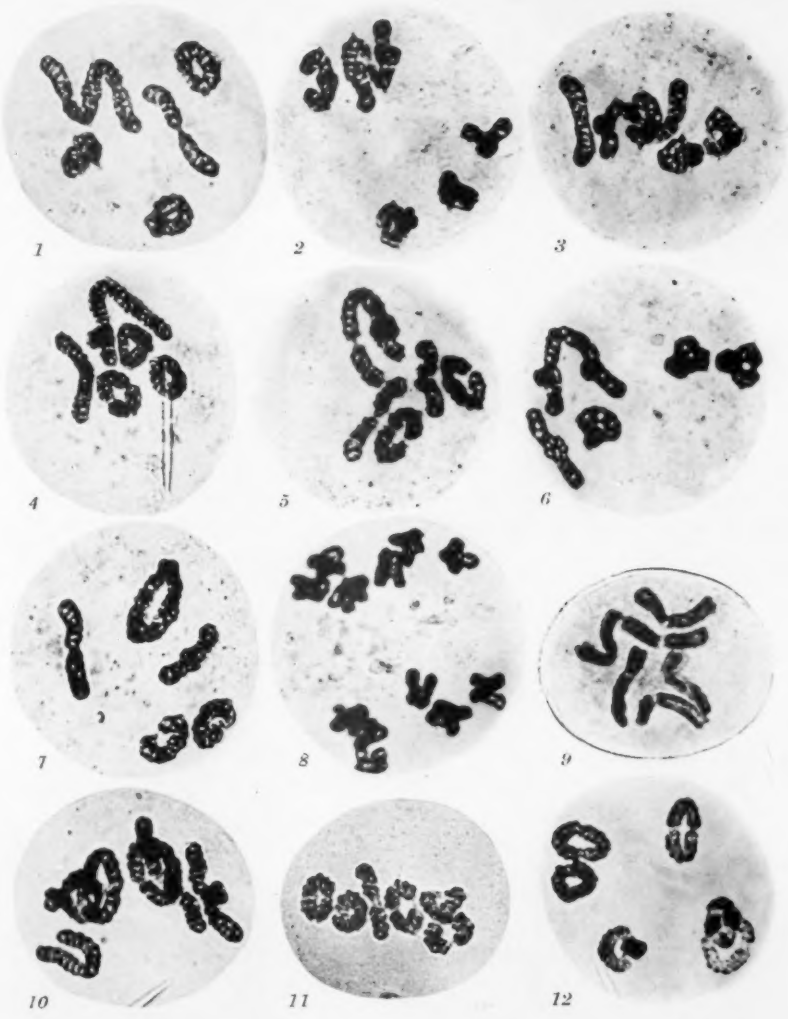
Figure 8.—Later anaphase of first meiotic division with 6 chromosomes at each pole. The chromatids of each homologue are attached only in the region of the spindle fiber.

Figure 9.—The haploid set of chromosomes in the microspore. Three of the chromosomes are approximately isobrachial, and three are heterobrachial.

Figure 10.—Meiotic chromosomes of *T. edwardsiana* 15, with 6 bivalents. Two ring bivalents are interlocked, and a rod bivalent is locked with a ring bivalent.

Figure 11.—Meiotic chromosomes of *T. bracteata*. Six bivalents were usually found, but chains and rings were found in rare cases. A ring of four chromosomes is shown at the right.

Figure 12.—Meiotic chromosomes of *T. edwardsiana* 15 with interlocked ring bivalents. Interlocked bivalents were found in most of the pollen mother cells of this plant.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pressed on a sheet of filter paper. In this way the excess fixing fluid was removed, and the cells were flattened sufficiently so that the chromosomes could be studied and photographed easily.

The chromosomes of normal diploid Tradescantias usually form ring- and rod-shaped figures at meiosis. The homologous chromosomes may be united by terminal or subterminal chiasmata. Occasionally three chiasmata are found in a single bivalent. The average chiasma frequency found in *T. bracteata* was 1.9, in *T. reflexa* 1.8, and in *T. gigantea* 1.7.

Interlocked bivalents (figures 10 and 12) have been found in all diploid species examined. In *T. bracteata*, interlocked bivalents were found in 20 percent of the pollen mother cells; in *T. reflexa* 15 percent, and in *T. gigantea* 40 percent of the pollen mother cells contained interlocked chromosomes.

In all these diploid species there are six pairs of chromosomes of which three have sub-median spindle-fiber constrictions and the other three have approximately median constrictions.

The arrangement of the chromosomes in tetraploid Tradescantias is of interest in comparison with the configurations found in segmentally interchanged diploids. An examination of the chromosomes in 73 pollen mother cells of a tetraploid, *T. occidentalis*, showed that about half of the chromosomes were paired as bivalents and about half as quadrivalents. The quadrivalents were found as rings or chains of chromosomes with terminal chiasmata. In about 80 percent of the quadrivalents, adjacent chromosomes passed to opposite poles. The normal tetraploids are fertile and 85–90 percent of the pollen is morphologically perfect.

T. edwardsiana is a well-marked and distinctive species, though only recently described. It is apparently limited to the Edwards Plateau in central Texas and morphologically seems to be more closely related to the northern species *T. pilosa* than to any other known species from Texas. Collections were made at two near-by points in the Bull Creek region, near Austin, two plants being collected at the first station and eighteen at the other. Two of the plants from the second station (numbers 2 and 15A) were characterized by four bivalents and a chain or ring of four chromosomes at meiosis. The other 18 plants were apparently normal diploids with 6 bivalent chromosomes. The interchange chromosomes were found as chains of four in most cases. The four chromosomes are united end to end by terminal chiasmata (figure 1) or by terminal and subterminal chiasmata (figures 2, 3, 4, 5, 6, and 7). In rare cases each of the three chiasmata in the chain may be subterminal. Closed rings with four chiasmata are comparatively rare (figure 7). The types and frequency of chiasmata found in the interchange complex are shown in table 1.

A total of 648 pollen mother cells was studied. In 550 cells, chains or

TABLE 1

Chiasma frequency in interchange complex of T. edwardsiana plant 15A. (Numbers in parentheses represent subterminal chiasmata.)

3	3(1)	3(2)	3(3)	4	4(1)	4(2)	N
158	228	125	12	6	14	7	550

rings of four chromosomes were found, while in 98 cells only bivalent chromosomes were observed. In four cases there were trivalent-univalent groups of chromosomes. The average chiasma frequency in the chains and rings is only 3.05. Thirty-two percent of the chiasmata are subterminal.

In the chain of four chromosomes, subterminal chiasmata are found between the first and second or between the third and fourth chromosomes (figures 2, 3, 4, 5, and 6) but are rare between the second and third chromosomes. This distribution of the subterminal chiasmata and the prevalence of chains rather than rings indicate that segmental interchange has occurred between heterobrachial chromosomes. The arrangement of the chromosome segments is presumably indicated by figure 13.

FIGURE 13.

The adjacent chromosomes in the chain may go to opposite poles (figures 1, 2, and 3), forming fertile combinations of chromosomes, or they may go to the same pole (figures 4, 5, 6, and 7), forming sterile combinations due to a deficiency of a chromosome segment. The two types of distribution occur with about equal frequency—266 fertile to 284 sterile.

The proportions of fertile and sterile types of segregation are partly dependent on the number and position of the chiasmata in the chains and rings. The chains and rings with only terminal chiasmata show a ratio of 108 fertile to 56 sterile types of segregation. In chains with one subterminal chiasma, the ratio was 110 fertile to 132 sterile; with two subterminal chiasmata the ratio was 48 fertile to 84 sterile, while the 12 chains or rings with 3 subterminal chiasmata were all of the sterile type. Of the 27 closed rings only 1 was of the fertile type with adjacent chromosomes passing to opposite poles.

TABLE 2

Regular and non-disjunctional segregation of chains of 4 chromosomes in relation to the presence of subterminal chiasmata.

NUMBER SUBTERMINAL CHIASMATA	REGULAR	NON-DISJUNCTIONAL	PERCENT NON-DISJUNCTIONAL
0	108	56	34
1	110	132	55
2	48	84	64
3		12	100

The sterility expected from the observed percentages of the sterile type of segregation in the ring or chain is about 50 percent. But in about 15 percent of the pollen mother cells of plants 15A, only bivalents are found, and the segregation of the two bivalents of the interchange complex must be considered. If the homologous chromosomes of each of these two bivalents pass at random to either pole, the percentage of sterility expected will not be changed. The percentage of fertile types of segregation is greatest in chains of chromosomes with no terminal chiasmata, so with even greater freedom of assortment in two bivalents, there might be an excess of fertile types among the bivalents which would increase the fertility somewhat above 50 percent.

Pollen sterility is also dependent on chromosome distribution. In about 90 percent of the pollen mother cells, six chromosomes were found at each pole at telophase (figure 8), but in about 10 percent of the cells the distribution was 5–7. The microspores with 5 chromosomes apparently do not develop; but 7 chromosomes have been observed in microspore divisions, indicating that these types may form good pollen grains. Lagging univalents were also found which would result in chromosome deficiencies. An examination of 600 pollen mother cells showed lagging univalents in 30 cases. Unequal chromosome distribution and lagging univalents should produce about 7 percent pollen sterility.

Pollen counts from anthers about to open indicate about 50 percent sterility—642 apparently normal pollen grains and 538 imperfect ones. In view of the variations in chromosome segregation and pollen sterility, the correlation between chromosome behavior and pollen sterility is very close.

Interlocking of chromosomes was found in the segmental interchange plants as well as in normal diploids. In about 10 percent of the pollen mother cells of plants 15A, the chain or ring was interlocked with a bivalent. A ring bivalent locked on a chain is shown in figure 3. Occasionally two bivalents are locked on a chain or ring. Interlocking of bivalents was also found, but occurs in only 5 percent of the pollen mother cells. The total amount of interlocking between non-homologous chromosomes in *T. edwardsiana* 15A is less than in other normal species and much less than in some of the plants of *T. edwardsiana* with 6 bivalents.

A plant of *T. edwardsiana* (15) with 6 bivalents was examined for comparison with the segmental interchange plant (15A). The number and types of chiasmata found in the 6 bivalents of plant 15 and in the 4 bivalents of plant 15A are shown in table 3.

The average chiasma frequency is the same for the bivalents of both plants, although the number of subterminal chiasmata is greater in 15A (33 percent) than in 15 (19 percent). In the rings and chains of plant 15A,

TABLE 3

Number of chiasmata in bivalents. (Number in parentheses are subterminal.)

		0	1	(1)	2	2(1)	2(2)	3(1)	N	AVERAGE
T. edwardsiana	15	2	120	51	303	124	4	14	618	1.7
T. edwardsiana	15A	4	28	22	47	47	8	12	168	1.7

the percentage of subterminal chiasmata is also about 33 percent, but the chiasma frequency is about 3, or 1.5 per bivalent.

Only 2 interlocked bivalents were found in 42 pollen mother cells of plant 15A, while, in plant 15, there were 148 interlocked bivalents in 103 pollen mother cells. Two interlocked ring bivalents and a ring locked around a rod are shown in figure 10. Another pollen mother cell of plant 15, with interlocked bivalents is shown in figure 12. In a few cases as many as 5 bivalents were found interlocked, and interlocking of three bivalents was found frequently. Occasionally a ring or rod bivalent was locked with a double ring bivalent, but it was impossible to determine whether or not the locking involved the internode with the fiber constriction. There was about 30 percent pollen sterility in plant 15, when grown in the greenhouse, which might indicate that the chromosome behavior is not entirely normal.

Chromosome rings and chains have also been found in a single plant of *T. reflexa*. This species is usually a tetraploid in the northern states (ANDERSON and DIEHL 1932), but several diploid plants from eastern Michigan were obtained through the courtesy of the Botanical Garden of the UNIVERSITY OF MICHIGAN. One of them, a peculiar looking plant with light, gray-blue flowers, had been collected at Dexter, Michigan. Like the two plants of *T. edwardsiana*, it was characterized by rings and chains of 4 at the reduction division. The average chiasma frequency was found to be 1.76 for the bivalent chromosomes and 3.84 for the rings and chains. Interlocking of bivalents was rare, only one case in 107 pollen mother cells, but interlocking of the ring or chain with a bivalent was observed in 15 pollen mother cells out of the 107.

In this Dexter plant, the rings were much more frequent than chains. Adjacent chromosomes were oriented towards the same pole in 83 percent of the 107 cells studied. About 90 percent sterility might be expected with such a segregation if the interchange complex is typical, but the pollen sterility is usually relatively low. Pollen counts from 98 different flowers, and on 16 different days, showed from 10 to almost 100 percent fertility. The general average of all pollen counts (from flowers collected on different dates) was 65 percent morphologically perfect pollen. Counts from individual anthers showed uniformity for individual flowers.

The chromosomes of the interchange complex in *T. reflexa* may be united by either terminal or subterminal chiasmata. A chain of four chromosomes united by terminal chiasmata is shown in figure 14A. In about 40 percent of the rings or chains, one or more subterminal chiasmata were observed. A typical ring with a subterminal chiasma is shown in figure 14B. This chiasma is peculiar in that it appears to form a loop, and the adjacent chromosome segments are at different levels in some cases. The asymmetrical arrangement of the chromosomes was found in many figures, and the position of the subterminal chiasma is typical. In many of the microspore divisions, one chromosome was considerably longer than the others.

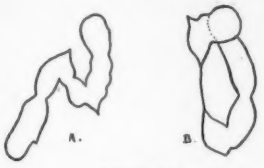

FIGURE 14.

In certain diploid species with 6 bivalents at meiosis, rings or chains of four chromosomes are very rarely found. Several such figures have been found in *T. edwardsiana* and in *T. bracteata*. A ring of four chromosomes from *T. bracteata* is shown in figure 11. The rings may be either double loops or circles. They seem to be essentially the same as those found in segmental interchange plants.

The rings and chains of 12 chromosomes in the closely related genus Rhoeo have been studied for a comparison with the rings of 4 in Tradescantia. According to DARLINGTON (1929), non-disjunction in the 12 chromosomes of Rhoeo occurs in about half of the pollen mother cells. One of the writers (SAX 1931) also found about 50 percent non-disjunction, but the counts were not based on many figures. According to GAIRDNER and DARLINGTON (1932), non-disjunction is about 30 percent in Rhoeo, but no data or references are given in support of this conclusion.

A careful study of 100 pollen mother cells of *Rhoeo discolor* showed that all 12 adjacent chromosomes were oriented towards opposite poles in only 21 cells. Single non-disjunction was found in 39 cells, double non-disjunction on opposite sides of the spindle was found in 30 cells, and double non-disjunction on the same side was found in 9 cells. Thus, in nearly 80 percent of the cells, there was non-disjunction in the ring or chain of 12 chromosomes. This amount of non-disjunction should result in about 80 percent pollen sterility. The pollen sterility actually found in an examination of about 1000 pollen grains was 86 percent, which is in accord with previous observations (SAX 1931).

The amount of non-disjunction is greater in rings than in chains. In 31 rings, 27 had double non-disjunction, and 2 had quadruple non-disjunction. Only 3 of the 31 rings were regular in chromosome arrangement. In 19 single chains 10 were regular; 8 had single non-disjunction, and 1 cell had double non-disjunction. The cases of single non-disjunction are, of course, accompanied by a second one if the chromosome distribution is 6–6. In 25 cells with two chains, 7 were apparently regular in chromosome arrangement and 18 showed either single or double non-disjunction. The single cell observed with three chains had an apparent regular orientation of chromosomes. It is evident that non-disjunction is influenced by chromosome arrangement. In Rhoeo, chromosome rings show non-disjunction in about 90 percent of the figures examined, while chains show non-disjunction in only 60 percent of the figures.

DISCUSSION

Chromosome ring formation resulting from segmental interchange is usually associated with pollen sterility due to non-disjunction of chromosome segments. If adjacent chromosomes pass to the same pole, the resulting microspores should be sterile owing to deficiency of chromosome segments; but if adjacent chromosomes pass to opposite poles, each microspore should have a complete chromosome complement and would be capable of further development. The percentage of non-disjunction in segmental interchange rings may be very low in certain genera and high in others. A comparison of the ring-forming chromosomes in different genera should throw some light on the cause of non-disjunction in chains and rings of chromosomes.

CLELAND (1929) has found that adjacent chromosomes pass to opposite poles in about 80 percent of the rings of *Oenothera Lamarckina*. Most of the chromosomes have approximately median fiber constrictions, and the 12 chromosomes of the ring are attached end to end.

In Datura, BLAKESLEE (1929) finds normal pollen fertility in plants with a ring of four chromosomes. He assumes that adjacent chromosomes in the ring always pass to opposite poles, a situation resulting in fertile combinations, but no data on chromosome distribution are given. The chromosomes of Datura have approximately median spindle-fiber constrictions, and homologous chromosomes are united only at the ends in both rings and bivalents.

Chromosome rings and chains in Zea have been found by MCCLINTOCK (1930), BURNHAM (1930, 1932), and by BRINK and COOPER (1931, 1932). In plants with a ring of four chromosomes, the pollen sterility is about 50 percent; with two rings, it is about 75 percent, as would be expected if adjacent chromosomes pass to the same pole or to opposite poles at ran-

dom. BURNHAM (1932) found that non-disjunctional and disjunctional segregation occur with about equal frequency. The attachment constrictions of Zea are more or less sub-terminal, and subterminal chiasmata are found in many of the rings.

PELLEW and SANSOME (1931) found that ring formation in Pisum is associated with about 50 percent pollen sterility, and in about half the cases examined, non-disjunction occurs in the ring, although no actual data on frequency of different types of segregation are presented. In the rings of four chromosomes, interstitial and subterminal chiasmata were frequently found. Two of the chromosomes appear to have sub-terminal fiber constrictions.

GAIRDNER and DARLINGTON (1932) find rings of four and six in Campanula. These writers state that, in the rings, the chromosomes were arranged non-disjunctionally in about 30 percent of the cells; but the published data (table 2) show 38 percent non-disjunction in rings and 65 percent in chains, or an average of 41 percent non-disjunction. The chromosomes of Campanula have approximately median fiber constrictions, and in both rings and bivalents the chromosomes are united only by terminal chiasmata.

Segmental interchange rings with relatively isobrachial chromosomes attached by terminal chiasmata seem to be more regular in disjunction than rings with heterobrachial chromosomes or rings with interstitial and subterminal chiasmata. The first group includes Datura with practically no non-disjunction, Oenothera with 20 to 30 percent non-disjunction, and Campanula with 30 to 40 percent non-disjunction. The second group includes Zea, Pisum, and Tradescantia, each with about 50 percent non-disjunction, and Rhoeo with about 80 percent non-disjunction.

In *Tradescantia edwardsiana*, the 4 chromosomes involved in the ring are apparently heterobrachial, as indicated by the position of the interstitial or subterminal chiasmata. In the non-disjunction types of chains, the short segments are oriented towards the poles, while the longer segments are frequently united by subterminal chiasmata (figures 3, 4, and 5). These configurations may be due to the greater repulsion between the long homologous segments. In Rhoeo, however, the adjacent long segments may pass to the same pole (SAX 1931, figures 8 and 9). But at least one of the four heterobrachial chromosomes is involved in cases of non-disjunction. Apparently the relative lengths of the chromosome segments in the ring do influence the regularity of disjunction, but the available data hardly warrant further discussion.

According to GAIRDNER and DARLINGTON (1932), chains disjoin less regularly than rings in both Campanula and Rhoeo. This conclusion will not hold for Rhoeo or Tradescantia and is of doubtful validity in Cam-

panula. The 13 elongated S-shaped chains in Campanula (table 2) are classed as non-disjunctional, but they may be regularly distributed so that adjacent chromosomes pass to opposite poles. Figures of these chromosome types in Campanula are not shown, and they have never been observed by the writers in either segmental interchange chains or in tetravalent chromosomes of Tradescantia. If these 13 chains are regular in distribution, the percentage of non-disjunction is greater in rings than in chains. In the interchange plant, *Tradescantia edwardsiana*, non-disjunction was found in all but one of the 27 rings observed, while in the 523 chains, the regular and non-disjunctional types were found in about equal proportions. Non-disjunction was found in 90 percent of the rings but in only 60 percent of the chains in Rhoeo. The greater regularity of disjunction in chains can be attributed to the greater flexibility in chromosome arrangement, which enables homologous segments to repel each other with greater frequency than is possible in rings.

The effects of rigidity in rings or chains are also clearly shown in the interchange complexes of *T. edwardsiana*. The chains and rings with no subterminal chiasmata were non-disjunctional in only 35 percent of the cells. With one subterminal chiasma, the percentage of non-disjunction was 55; with two subterminal chiasmata, it was 64 percent; and when there were three subterminal chiasmata in the chain or ring, all figures were non-disjunctional. It is clear that the greater rigidity of the chains or rings caused by subterminal chiasmata increases the proportion of non-disjunction.

GAIRDNER and DARLINGTON have suggested that the rigidity caused by interstitial chiasmata in Pisum is the cause of the frequent non-disjunction (50 percent) in the interchange rings. The data reported above demonstrated that interstitial chiasmata do have an important effect upon the percentage of non-disjunction in Tradescantia. But the presence of interstitial chiasmata is only one of several factors which influence the type of segregation. In Rhoeo with no subterminal chiasmata, non-disjunction is found in about 80 percent of the cells. In the segmental interchange *T. edwardsiana*, about 60 percent of the rings and chains have subterminal chiasmata, but non-disjunction is found in only half of the pollen mother cells. Only 40 percent of the interchange rings and chains in *T. reflexa* have subterminal chiasmata, but non-disjunction occurs in over 80 percent of the cells. The quadrivalents of *T. occidentalis* have only terminal chiasmata, and adjacent chromosomes pass to the same pole in only 20 percent of the cells. In Rhoeo and *T. reflexa* with high non-disjunction, the arms of certain chromosomes vary considerably in length. In *T. edwardsiana*, these differences are less extreme, and in *T. occidentalis* the chromosomes which form quadrivalents usually have approximately median constric-

tions. Non-disjunction is increased by ring formation, by the position of spindle fibers and relative lengths of chromosome segments in the ring or chain, and by the presence of interstitial or subterminal chiasmata.

The percentage of non-disjunction in segmental interchange plants is usually closely associated with the degree of pollen sterility. The Dexter plant, *T. reflexa*, is exceptional, since it has over 80 percent non-disjunction in the rings and chains and an average of 65 percent pollen fertility. The high degree of fertility may be due to interchange involving the translocation of all, or almost all, the one chromosome to a segment of another, so that the constitution of the four chromosomes would be Aa, aB, Bb, and bBA. Such an interchange complex would produce the very long chromosomes observed in rings and in microspore divisions, and would account for the high pollen fertility.

About 30 percent pollen sterility was found in a diploid, *T. edwardsiana*, with 6 bivalents. This plant may have unequal segmental interchange involving segments too short to pair, so that only bivalents are produced. If one translocated segment is too small to have a lethal effect, the pollen sterility would be only 25 to 30 percent. BURNHAM (1932) finds only 25 percent sterility in a case of unequal interchange in Zea and has suggested that only bivalents might be formed if only small segments were interchanged. The pollen sterility could also be accounted for on the assumption that one chromosome is deficient for a segment which is translocated to a non-homologous chromosome.

Segmental interchange reduces crossover frequency in Drosophila (DOBZHANSKY 1931), but in Tradescantia, chiasma frequency is about the same in interchange rings and bivalents. The average chiasma frequency is 1.7 for the four bivalents of *T. edwardsiana* and 2.9 for the four interchange chromosomes including the 98 bivalents. However, the rings and chains probably include the two "pairs" of heterobrachial chromosomes, and three of the four bivalents are isobrachial, so that the potential amount of chiasma formation may differ in the two classes. In the *T. reflexa* from Dexter, the average chiasma frequency is 1.76 for bivalents and 3.84 for rings, or a slightly higher frequency in the rings, on the basis of bivalents. The percentage of subterminal chiasmata per chromosome in chains and rings is about the same as it is in the bivalents in both species of Tradescantia.

In an earlier paper (SAX 1931), it was suggested that segmental interchange may result from interlocking of bivalent chromosomes, although it is known that the action of X-rays produces translocations in the mature sperm of Drosophila and in the pollen of Zea. The relative frequency of interlocked bivalents in Oenothera, Campanula and Tradescantia does suggest a correlation between interlocking and segmental interchange,

but it is possible that interlocking is due primarily to interchange of segments too short to cause the formation of chains or rings. The attraction between these short segments might bring the chromosomes in closer contact at the time of pairing, so that interlocking of non-homologous chromosomes would occur. In rare cases chains or rings of four chromosomes have been observed in Tradescantia species which usually have six bivalents. Over ten such figures have been observed in *T. edwardsiana* and *T. bracteata*, one of which is shown in figure 11. These figures suggest that segmental interchange chromosomes may originate at meiosis owing to breaks in interlocked bivalents, but it is also possible that they represent segmental interchange types of earlier origin which involve such short segments that ring and chain formation rarely occurs.

The interlocking of bivalents is of interest in relation to chiasma formation and the mechanism of crossing over. As CATCHESIDE (1931) has pointed out, interlocking of bivalents can occur only at alternate internodes if chiasmata are formed by alternate opening out of pairs of chromatids in reductional and equational planes. If chiasmata are the result of previous crossovers, then one bivalent could be locked with another at any internode. GAIRDNER and DARLINGTON (1932), in their discussion of interlocking in Campanula, state that "Interlocking does not seem to occur here (or in any other organism) between the chromatids of paired or unpaired chromosomes. Yet such interlocking should occur both with and without terminalization if the chromatids ever separate equationally at diplotene."

If chiasmata are caused by alternate opening out of pairs of sister and non-sister chromatids, interlocking could occur only at internode 2, as shown in figure 15 and never at internodes 1 or 3.

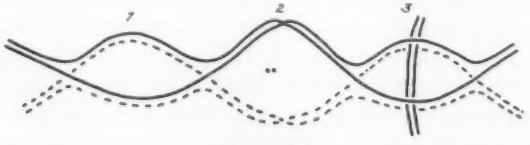

FIGURE 15.

Interlocking of one chromosome between the chromatids of a bivalent, as shown at internode 3, could not occur because the closely paired non-sister threads could move apart in an equational plane only with difficulty, owing to the interlocked pair of chromatids between them. The interlocking at pachytene would prevent a close association of non-sister threads in the region of interlocking, and a reductional opening out of paired sister chromatids would have been initiated at the earliest pachytene stage of pairing. We would expect, then, that interlocking would in-

volve only the reductional internodes and that interlocking between chromatids would never occur.

In almost all of the described cases of interlocked bivalents, only one internode is found in each pair of chromosomes, and the two chiasmata are usually terminal in each bivalent. This type of association would be expected where chromosomes have an approximately median fiber attachment point, because interlocking could occur only at the median internode, unless more than three internodes are formed, or unless three chiasmata are formed distal to the spindle fiber. Terminalization or breaks in the chiasmata could account for ring bivalents at diakinesis and metaphase.

If chiasmata are the result of previous crossovers and only sister chromatids are paired at early diplotene, as DARLINGTON and others assume, the interlocking of bivalents could occur at any internode. According to GAIRDNER and DARLINGTON, Campanula bivalents have from two to six chiasmata at diplotene. These chiasmata are said to be formed "interstitially and at random." CATCHESIDE (1931) found that three is the most usual number of chiasmata in Oenothera bivalents at diplotene, but as many as four or five may be formed. As many as four or five chiasmata have been observed in Tradescantia bivalents at late diplotene stages. With such a high chiasma frequency at diplotene in these genera, there should be many cases of distal interlocking of bivalents. In Campanula, proximal interlocking occurs in about 20 percent of the pollen mother cells while "distal interlocking seems to be much rarer." The apparent contact of rings is interpreted as distal interlocking with the distal internode terminalized to such an extent that no distal loop can be observed (GAIRDNER and DARLINGTON, figures 16 C, E, G, and J). Proximal interlocking of bivalents is frequently found in Oenothera, but distal interlocking is very rare and has never been clearly demonstrated (CATCHESIDE 1931). Tradescantia bivalents with two internodes are, in rare cases, locked with a rod bivalent, but it was impossible to determine whether or not the locked internode was proximal or distal to the spindle fiber. In all these genera the bivalents usually take the form of rings with terminal or subterminal chiasmata. On either theory of chiasma formation, practically all the interlocking of such ring bivalents would appear to involve proximal internodes. If all but two chiasmata pass off the ends of each bivalent by terminalization, only proximal interlocking could occur. But if terminalization simply accumulates the chiasmata at the ends of the chromosomes, as GAIRDNER and DARLINGTON assume to be the case in Campanula, then distal interlocking should be at least as frequent as proximal interlocking if chiasmata are the result of previous crossovers. If chiasmata are caused by alternate pairing of sister and non-sister chromatids, distal interlocking

should be rare, which is the case in all the genera with interlocked bivalents.

SUMMARY

Chains or rings of four chromosomes were found in two diploid plants of *Tradescantia edwardsiana*. The average chiasma frequency in the segmental interchange complex was 2.9 including potential chains, as compared with an average chiasma frequency of 1.7 for the four bivalents. The prevalence of chains and the position of subterminal chiasmata in the rings and chains are attributed to the relative lengths of the chromosome segments which pair. Non-disjunction is more frequent in rings than chains, and is more frequent in chains with one or more subterminal chiasmata than in chains with only terminal chiasmata. Non-disjunction in rings and chains was about 50 percent, which is in accord with the degree of pollen sterility found.

About 30 percent pollen sterility was found in a plant of *T. edwardsiana* with 6 bivalent chromosomes. This sterility is attributed to segmental interchange involving unequal segments, or to a translocation resulting in deficiency and duplication of chromosome segments. Interlocking of some of the bivalent chromosomes was found in almost all pollen mother cells of this plant.

In a diploid *T. reflexa* from Dexter, Michigan, a ring of four chromosomes and four bivalents was usually found at meiosis. The average chiasma frequency in the rings or chains was 3.84 and was 1.76 for the bivalents. Non-disjunction was found in 78 percent of the rings and chains, but about 60 percent of the pollen was good. This degree of fertility is attributed to a segmental interchange which is in part a duplication.

Chiasma frequency in bivalents of segmental interchange plants is about the same as in normal diploids. Segmental interchange produces only a slight reduction in chiasma frequency and does not materially affect the percentages of terminal and sub-terminal chiasmata.

An analysis of non-disjunction in the rings and chains of 12 chromosomes in Rhoeo shows that non-disjunction is much more frequent in rings than in chains. The total amount of non-disjunction found was 79 percent, which is in accord with the pollen sterility found.

A comparison of the amount of non-disjunction reported in Oenothera, Datura, Campanula, Zea, Pisum, and in Tradescantia and Rhoeo, shows that the percentage of non-disjunction is dependent, in part, at least, on the proportional lengths of homologous segments in the chromosomes of the segmental interchange complex and the proportion of interstitial or subterminal chiasmata. Greatest regularity in disjunction of rings or chains is found where the chromosomes are isobrachial and are attached only by terminal chiasmata.

Interlocked bivalents are frequently found in Tradescantia and in other genera in which segmental interchange rings are found. Breaks and a reunion of non-homologous segments in such interlocked bivalents might account for the segmental interchange rings. Chains or rings of four chromosomes are occasionally found in Tradescantia plants which normally have only six bivalents. The prevalence of interlocked bivalents in genera where segmental interchange is found may, however, be due to interchange of chromosome segments too short to insure pairing in chains or rings, but long enough to cause some interlocking of chromosomes at the time of pairing.

The prevalence of proximal interlocking of non-homologous chromosomes in Oenothera, Tradescantia, and Campanula, is in accord with the assumption that chiasmata are the result of alternate pairing of sister and non-sister chromatids.

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NINE INDEPENDENTLY INHERITED AUTOSOMAL FACTORS IN THE DOMESTIC FOWL[1]

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CHROMOSOMAL COMPLEX

The question of the exact number of chromosomes in the cells of the domestic fowl, *Gallus domesticus*, still remains an unanswered one. As cytological material the cells of this species have proved to be unsatisfactory. HANCE (1926) who has given considerable time to the cytology of the domestic fowl states that much of the difficulty with this form has been due to the failure of the fixation chemicals to penetrate the tissue.

Most of the chromosomes of the fowl are large enough to be easily recognized but a few are very small. In these very small chromosomes is the source of the difference of opinion as to the exact count. STEVENS (see BORING 1923) gave the unreduced number as 34, SHIWAGO (1924) placed it at 32 and HANCE (1924) obtained a count of 34 or 35. All workers have recognized the sex chromosome which is the largest member of the complex. This chromosome is paired in the male but there remains a difference of opinion as to whether it is unpaired or has an unlike mate in the female.

Considerable attention (SEREBROVSKY and WASSINA, 1926), (WARREN 1928), (HERTWIG and RITTERHAUS 1929) has been given to the mapping of the factors of the sex chromosome since they are readily recognized by their crisscross type of inheritance. However, very little study has been made of the autosomal factors. Regardless of the fact that there remains a question as to the exact number of autosomes in the fowl the problem of linkage relations justifies some consideration. The only well established case of linkage between autosomal factors is that for rose comb and creeper (SEREBROVSKY and PETROV 1928). The large number of known workable characters in the fowl offer material for study of the linkage relations in this species.

CHARACTERS CONSIDERED

The writer has selected nine fairly well known characters of which the contrasted expressions were for the most part recognizable in the day-old chick. This latter condition is especially desirable since there is avoided the losses from early mortality and the expense of carrying the birds to maturity. Some of the characters have been included not because of their adaptability for linkage studies but because they are part of the make-up of the breeds used.

[1] Contribution No. 67 from the Department of Poultry Husbandry.

The dominant members of the allelomorphic pairs were, naked-neck, rumpless, white skin, leg-feathering, rose comb, pea comb, crest, polydactyly, and dominant white. With the exception of crest and white skin these characters were all recognizable in the day-old chick. Much of the data were obtained from the examination of embryos taken from the shell

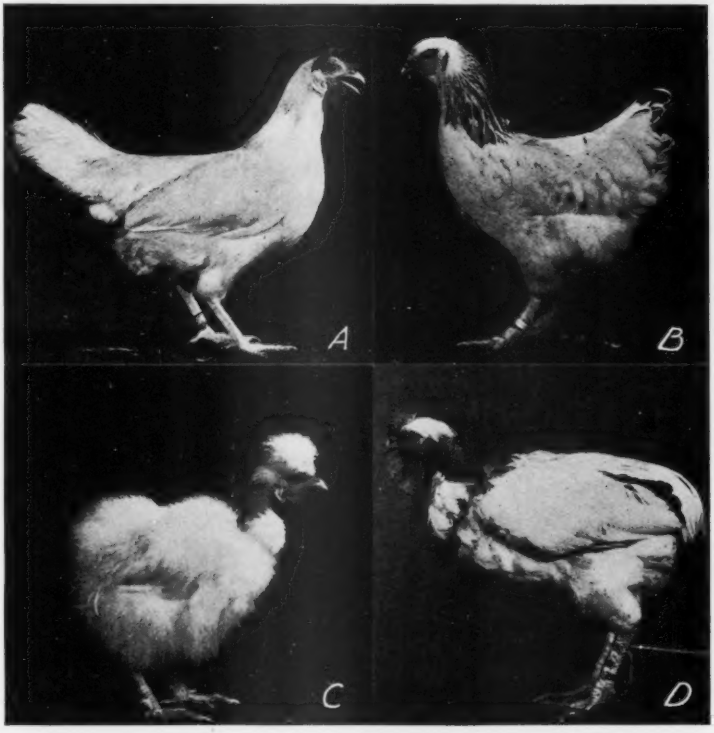

FIGURE 1.—The above reproduced birds show eight of the characters considered in this paper as follows: Naked-neck, C and D; rumplessness, C and D; leg-feathering, B and C; rose comb, D; pea comb, B; polydactyly, C; crest, C; and dominant white, A. Differences in skin color could not be shown by means of photographs.

on the eighteenth day of incubation since this eliminated much of the work involved in individual pedigreeing of the chicks. In cases where the characters could not be recognized at hatching the individuals were described at eight and twenty-four weeks of age.

NAKED-NECK

The naked-neck individuals are characterized by the complete absence of feathers on the neck. There is also a tendency to slightly restrict the

feather tracts in other regions of the body. Individuals exhibiting this character show excellent viability and it is expressed very early in embryonic development. The ease of classification makes it a very excellent character for genetic studies. This character has been shown by DAVENPORT (1914) and CREW (1922) to behave as a simple dominant. Table 1 presents the results of backcrossing F_1 birds to the recessive normal condition. This table includes results both from chicks and adults. Most of those included in the chick classification were recorded on the eighteenth day of incubation. This section of the table also includes a few chicks that were described at the day-old stage but died soon after. In case the character is of low viability the chick section of the table should give better ratios than the adult section since it includes individuals which do not have sufficient vigor to escape from the shell and thus would be eliminated from the adult classification.

TABLE 1

Backcross of males and females heterozygous for naked-neck to recessive normals.

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		NAKED	NORMAL	NAKED	NORMAL	NAKED	NORMAL	NAKED	NORMAL
1061M	♂	38	39	57	38	32	28	38	35
1074M	♂	34	36	41	41	47	38	53	52
1099M	♂	47	55	69	72	102	61	76	92
1102M	♂	25	31	30	31	30	16	17	9
1143M	♂	..	..	..	..	25	28	39	38
1144M	♂	59	48	48	46	22	20	23	28
1145M	♂	..	..	..	..	59	58	52	65
1147M	♂	16	23	22	26	..	..	..	..
1148M	♀	60	72	43	48	..	..	..	..
1149M	♂	..	..	..	..	51	44	49	47
1150M	♂	21	27	27	24	..	..	..	..
1159M	♀	..	..	..	..	18	11	18	14
Total		300	331	337	326	386	304	365	380

Grand Total—1388 Naked to 1341 Normals

The results show no sexual dimorphism in the expression of the character. The totals of the adult classification approach very closely to the expected 1 to 1 ratio with 637 naked to 657 normals. The chicks showed a preponderance of nakeds, 751 to 684 normals. The grand total gave 1388 naked to 1341 normals.

Rumplessness

The character is a well known dominant in poultry. DUNN (1925) found that this condition appears occasionally in a non-heritable form in chickens. Genetically it behaves as a simple dominant. The primary morphological

change in the rumpless fowl is the absence of the five free caudal vertebrae and the pygostyle. The loss of this portion of the skeleton causes the oil gland and most of the larger tail feathers to be absent.

Table 2 gives the results of backcrossing individuals heterozygous for rumpless to normals. As is the case of all tables in this publication, the chick section of the table includes individuals that were removed from the shell on the eighteenth day of incubation. The very close approximation of a 1 to 1 ratio in both the chick and adult sections of the table indicates that this character in no way handicaps the chick during development. The totals for the combined results were 798 rumpless to 833 normals. The rumpless condition may be recognized by touch as early as the eighteenth day of incubation.

TABLE 2

Backcross of males and females heterozygous for rumplessness to recessive normals.

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		RUMPLESS	NORMAL	RUMPLESS	NORMAL	RUMPLESS	NORMAL	RUMPLESS	NORMAL
1061M	♂	36	41	56	39	33	27	39	34
1074M	♂	30	40	38	44	55	30	51	54
1102M	♂	25	31	19	42	27	19	11	15
1143M	♂	..	..	..	..	24	29	37	40
1144M	♂	43	64	49	45	21	21	24	27
1147M	♂	15	24	24	24	..	..	..	..
1148M	♀	67	65	50	41	..	..	..	..
1159M	♀	..	..	..	..	11	18	13	19
Total		216	265	236	235	171	144	175	189

Grand Total—798 Rumpless to 833 Normals

Skin color

The white and yellow skin colors characteristic of various breeds of poultry constitute an allelomorphic pair of autosomal characters. White skin has been found to be dominant to the yellow. Since the difference is usually more easily detected in the shank the factor is sometimes referred to as one influencing shank color rather than skin color. Black pigmentation found in the shanks of some breeds interferes with classification with respect to the skin color factor. An examination of the bottom of the foot of birds carrying the dark shanks will usually make it possible to determine its skin color. Skin color cannot be accurately determined until the chick is beyond a month of age. The xanthyophyll pigment which gives the shank its yellow color fades in heavy producing females and is not well developed in chicks reared on feeds deficient in vitamin A. Errors of classification from these sources are not great and the ideal age at which classi-

fication may be made is just before the bird reaches sexual maturity. LAMBERT and KNOX (1927), DUNN (1925) and PUNNETT (1922) have published material on the inheritance of this character.

In table 3 are presented the data upon the segregation of skin color factors. Since classification cannot be made at hatching the chick section of the table has been omitted. The totals of 481 white skin to 477 yellow give a very close approach to a 1 to 1 ratio.

TABLE 3

Backcross of males and females heterozygous for white skin to recessive yellow skin.

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION			
		FEMALES		MALES	
		WHITE	YELLOW	WHITE	YELLOW
1061M	♂	28	26	21	37
1074M	♂	38	32	41	41
1099M	♂	50	52	86	55
1144M	♂	50	41	35	40
1146M	♂	29	32	29	40
1147M	♂	12	8	14	22
1150M	♂	25	23	23	28
Total		232	214	249	263

Grand Total—481 White Skin to 477 Yellow Skin

Leg-feathering

Some of the most comprehensive studies of this character are by PUNNETT and BAILEY (1918), SEREBROVSKY (1926), DUNN and JULL (1927) and LAMBERT and KNOX (1929). There is rather general agreement among them that the more sparse feathering of the legs found in the Langshan is due to a single factor difference while the heavy leg-feathering found in the Cochins and other Asiatic breeds is due to two pairs of factors. The two factors are dominant and cumulative in effect. This character is fully expressed in the day-old chick. Slight degrees of feathering may be more easily detected in the newly-hatched chick since the feathers may be lost by accident during growth.

The data in table 4 are based upon leg-feathering as it is found in the Light Brahma and Silky Bantam breeds. In these two breeds the leg-feathering is of the heavy type. The results are from backcrossing heterozygous individuals to non-feathered mates. The second column indicates the sex of the heterozygote. Males 1041M, 1061M, and 1149M were smooth-legged birds and mated to heterozygous feathered females which were the offspring of a Light Brahma male. Male 1079M was heterozygous for the Light Brahma type of feathering. Males 1144M and 1147M were heterozygous for the Silky Bantam leg-feathering. Males 1099M, 1101M, and

TABLE 4

Backcross of males and females heterozygous for leg-feathering to recessive normals.

MALE NUMBER	HETERO-ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		FEATHERED	NORMAL	FEATHERED	NORMAL	FEATHERED	NORMAL	FEATHERED	NORMAL
1041M	♀	64	79	79	76	..	..	..	..
1061M	♀	33	36	48	42	29	31	39	34
1079M	♂	..	..	..	..	49	41	40	32
1099M	♂	53	49	64	77	84	79	82	86
1101M	♀	..	..	..	..	31	67	26	55
1102M	♂	19	37	13	48	13	33	4	22
1144M	♂	47	60	53	41	15	27	14	37
1147M	♂	15	24	14	34	..	..	..	..
1149M	♀	..	..	..	..	18	77	33	63
Total		231	285	271	318	239	355	238	329

Grand Total—979 Feathered to 1287 Normals

1102M were all heterozygous F_2 segregates originating from the Light Brahma.

An examination of table 4 shows that in all matings the number of feathered-legged individuals was only equal to or less than that for smooth-legged ones. This would indicate that our material carried only a single factor for leg-feathering since on a cumulative two-factor basis it would be expected that the number of feathered would exceed the smooth.

Rose comb

This well known character requires no extensive description since it was one of the characters earliest studied in poultry. At hatching it is easily distinguished from its allelomorph, single comb.

BATESON early found that rose comb behaved as a simple dominant toward single. Results in table 5 showing the offspring from a backcross of

TABLE 5

Backcross of males and females heterozygous for rose comb to recessive single comb individuals.

MALE NUMBER	HETERO-ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		ROSE	SINGLE	ROSE	SINGLE	ROSE	SINGLE	ROSE	SINGLE
1074M	♂	37	33	37	45	44	41	58	47
1079M	♂	..	..	..	..	45	45	42	30
1101M	♀	..	..	..	..	41	57	43	38
1144M	♂	56	51	46	48	22	20	25	26
Total		93	84	83	93	152	163	168	141

Grand Total—496 Rose to 481 Single.

heterozygous individuals to those having single combs are in agreement with this view. The very close approximation of a 1 to 1 ratio indicates the good viability of the character. The similarity of results in the adult and chick section of the table shows that the character may be accurately recognized at hatching.

Pea comb

This character was also early studied. This gene in combination with that for rose produces a distinct type of comb called walnut. The fact that the double dominant condition may be recognized has made the cross of rose by pea a classic experiment as an example of a di-hybrid cross. Pea comb has always been listed as dominant to single comb and the dominance is practically perfect when the single comb breed is a small combed variety as is true in most heavy breeds. However, in crosses with the larger combed Mediterranean breeds the adult condition of the comb is much more like a single than a pea. The comb of the heterozygous bird is usually of a lopping single type carrying ridges on the side. There is seldom any difficulty in recognizing the heterozygote but the comb here is more like that of the single parent than that with the pea comb.

In table 6 the grand total shows a slight shortage of pea combs since the ratio is 920 pea to 1037 single. The totals for the two sections show that the shortage is all accounted for in the chick section of the table. The adult section totals 406 peas to 411 singles while the totals for the chick section are 514 to 626 singles. There is some variation in the combs of chicks heterozygous for pea and in some cases they might at that time be classed as singles. The genes for pea comb used in these studies were derived from the Light Brahma breed.

TABLE 6

Backcross of males and females heterozygous for pea comb to single comb individuals.

MALE NUMBER	HETERO-ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		PEA	SINGLE	PEA	SINGLE	PEA	SINGLE	PEA	SINGLE
1041M	♀	66	77	67	88	..	..	..	..
1061M	♀	36	33	42	48	..	..	..	..
1099M	♂	65	37	69	72	76	87	83	85
1101M	♀	..	..	..	..	26	36	24	29
1102M	♂	30	26	31	30	19	27	10	16
1143M	♂	..	..	..	..	25	28	36	38
1145M	♂	..	..	..	..	52	65	55	61
1149M	♀	..	..	..	..	38	57	39	57
1159M	♀	..	..	..	..	18	22	13	18
Total		197	173	209	238	254	322	260	304

Grand Total—920 Pea to 1037 Single

Crest

The crest characterized by elongated and more erect feathers on the head was derived from the Silky Bantam. The various workers (BATESON and SAUNDERS 1902, DAVENPORT 1909, BARFURTH 1911, DUNN and JULL 1927), have all placed this character on a monofactorial basis. The data here are rather limited but as shown in table 7 substantiate this view. The totals of 108 crested to 124 normals show a slight deficiency in the crested class.

TABLE 7

Backcross of heterozygous crested males to normal females.

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION			
		FEMALES		MALES	
		CREST	NORMAL	CREST	NORMAL
1144M	♂	48	46	35	42
1147M	♂	10	13	15	23
Total		58	59	50	65

Grand Total—108 Crested to 124 Normals

Polydactyly

Although considerable study has been given this character its mode of inheritance has not been completely analyzed. Investigators (DUNN and JULL 1927, PUNNETT and PEASE 1929) have agreed that it is probably on a simple monofactorial basis. PUNNETT and PEASE have suggested that an inhibitor may interfere with its regular segregation since four-toed individuals sometimes transmit the five-toed condition. The extra-toed condition has been found to be dominant to the more usual four-toes. Polydactyly as here considered was introduced through the Silky Bantam.

The results in table 8 show a large shortage of polydactyls. The totals are 167 polydactyls to 259 normals. On a monofactorial basis equal num-

TABLE 8

Backcross of males and females heterozygous for polydactyly to normal individuals.

MALE NUMBER	HETERO-ZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		POLY-DACTYLY	NORMAL	POLY-DACTYLY	NORMAL	POLY-DACTYLY	NORMAL	POLY-DACTYLY	NORMAL
1144M	♂	46	61	33	60	19	23	19	32
1147M	♀	11	28	19	30	..	..	..	..
1149M	♀	..	..	..	..	9	14	11	11
Total		57	89	52	90	28	37	30	43

Grand Total—167 Polydactyls to 259 Normals

bers would have been expected. The preponderance of normals occurs in each of the three matings.

Dominant white

Dominant white plumage which is characteristic of the White Leghorn breed is a well known color factor in the fowl. Its dominance to color is incomplete since some black flecks or dinginess usually occur; however, the heterozygous individual is always predominantly white.

The totals given in table 9 show a slight preponderance of whites, there being 674 whites to 627 coloreds.

TABLE 9

Backcross of males and females heterozygous for dominant white to colored individuals.

MALE NUMBER	HETEROZYGOUS SEX	ADULT DESCRIPTION				CHICK DESCRIPTION			
		FEMALES		MALES		FEMALES		MALES	
		WHITE	COLOR	WHITE	COLOR	WHITE	COLOR	WHITE	COLOR
1101M	♀	..	..	..	..	87	73	60	74
1143M	♂	..	..	..	..	31	22	41	33
1145M	♂	..	..	..	..	72	45	72	45
1146M	♂	35	26	31	38	..	..	..	..
1150M	♂	30	24	29	32	..	..	..	..
1159M	♀	..	..	..	..	38	33	27	38
1181M	♂	..	..	..	..	22	38	18	28
1184M	♂	..	..	..	..	37	42	44	36
Total		65	50	60	70	287	253	262	254

Grand Total—674 White to 627 Colored

TESTS FOR ASSOCIATION OF CHARACTERS

The results on tests for linkage relations were obtained from crosses of birds carrying in varying combinations the characters considered. Usually three or more were considered at a time but for the sake of clarity in presentation the tabulation has been in pairs. In as much as the purebred females usually produce better than the crossbreds, the backcrossed heterozygote has usually been a male. An additional advantage of making the cross in this direction is that in instances where it was difficult to obtain combinations desired, a single multiple heterozygote of the male sex when obtained would give as many offspring as several such females. In cases where the female has been the heterozygote it has been indicated in the table.

All data presented are from backcrosses of multiple heterozygotes to multiple recessives. Both chick and adult descriptions were used. The chick descriptions apply to day-old chicks and embryos taken from the shell on

the eighteenth day of incubation. Skin color and crest could not be recognized in day-old chicks. Both repulsion and coupling were employed for testing for linkage. There remains some question as to the number of factors involved in the inheritance of leg-feathering and polydactyly. If more than one pair of factors determine the expression of the character the linkage reactions will be affected. However, if one of the genes involved were at all closely associated with a factor for which a test is being made, the linkage should be detectable.

In making tests for linkage one is immediately confronted with the question of what should be considered as a minimum number of individuals necessary for establishing the fact of the existence of independent assortment. In a survey of this nature it is desirable to confine the numbers to the dependable minimum in order to cover as much ground as possible.

At the initial stages of a study of this kind we can probably say that cases of crossing over of 40 percent or more are of little value as well as very difficult to definitely establish. Such loose linkages are of little use in mapping the genes of the chromosomes and can probably be studied to an advantage only when linkage groups of the fowl are better known.

By use of the formula $0.6745\sqrt{p \cdot q/n}$ for calculation of the probable error, it is found for cases of crossing over of less than 40 percent, a total of 182 individuals is sufficient for establishing the fact that the deviation is not due to chance. This calculation is based upon the assumption that a deviation of 4 times its probable error is significant. The numbers required increase very rapidly as the percentage of crossing over increases; for example, crossing over of 45 percent requires 730 individuals to be assured that the deviations are not due to chance. Since the detection of linkages where the crossing over percentage is less than 40 is desired we have considered the total of 200 as sufficient for the establishment of the fact of independent assortment. In most cases our numbers are considerably beyond the value suggested.

PREVIOUSLY REPORTED LINKAGES

There seems to be only one well supported case of autosomal linkage in the fowl. The linkage of rose comb and creeper was reported by SEREBROVSKY and PETROV (1928). Creeper is characterized by a shortening of the leg bones. Their preliminary results indicated that there was approximately 8 percent of crossing over between these two factors. Later results by these same authors (1930) placed the percentage of crossing over at 10. However, the more extensive data of LANDAUER (1931) indicate that there is less than 1 percent of crossing over between creeper and rose.

The second case of linkage in the fowl was reported by DUNN and JULL (1927) and later by DUNN and LANDAUER (1930). They found evidence for

linkage of dominant white and cerebral hernia. The difficulty of establishing this case lies in the irregular genetic behavior of cerebral hernia. They tentatively placed dominant white and cerebral hernia 2 or 3 units apart on the same chromosome.

DUNN and JULL (1927) also found some evidence for the linkage of polydactyly and dominant white. Their data were very limited, however, and the writer's own data (table 10) showed no evidence for any association of these two factors. DUNN and JULL also suggested the linkage of crest and rose comb. SEREBROVSKY and PETROV (1930) place these two factors in the same chromosome. However, the more critical results of JULL (1930) failed to substantiate the earlier findings.

SEREBROVSKY and PETROV (1930) placed creeper, rose comb, dominant white and crest all in the same linkage group. This grouping seems to be as yet rather poorly supported since SEREBROVSKY himself found no evidence for the linkage of dominant white and rose and only evidence of very loose linkage of creeper and dominant white. JULL's failure to find evidence for linkage of crest and rose removes another from the group.

SEREBROVSKY and PETROV also placed blue and naked-neck in the same linkage group with 41.7 percent of crossing over between them. His numbers, 252 non-crossovers to 173 crossovers, were large enough so that the difference between the two classes may be taken as indicative of linkage. However, the writer has data upon this same relationship which fail to agree with those of SEREBROVSKY and PETROV. My own totals were 190 non-crossovers to 204 crossovers and here the difference is not significant. SEREBROVSKY and PETROV have also reported linkage between polydactyly and split comb with 33 percent of crossing over.

LINKAGE RESULTS

Table 10 presents the condensed results of the linkage studies.[1] In this table are given only the totals in the non-crossover and the crossover classes. These sums are separated by a dash, the non-crossovers being followed by the crossovers. By following down, and from left to right, through the table one may locate each combination tested. In three combinations where our own data were incomplete, the results of SEREBROVSKY and PETROV have been included. These results are marked with an asterisk.

The probable errors have not been given but in no case is there sufficient deviation from a 1 to 1 ratio to be indicative of linkage. Table 11 was arranged in order that the significance of the deviations could be checked upon by observation. The values here were arrived at by calculating the

[1] Detailed tables showing age at classification of the characters, the sex in which the test for crossing over was made, and whether the test was for coupling or repulsion are on file at the BROOKLYN BOTANIC GARDEN and may be obtained for examination on request.

TABLE 10

List of character combinations tested for linkage. The first number in each space is the total of non-crossovers and the other the total of crossovers.

	DOMINANT WHITE	POLY-DACTYLY	CREST	PEA COMB	ROSE COMB	LEG-FEATHERING	WHITE SKIN	RUMPLESS
Naked Neck	304–308	187–239	125–107	664–721	348–374	656–679	398–430	890–827
Rumpless	194–193	199–181	112–122	165–186	353–369	280–292	250–236	..
White Skin	161–148	112–110	107–115	120–123	163–155	217–248	..	..
Leg-feathering	157–137	199–182	115–119	727–697	325–310	..	..	..
Rose Comb	194–183	148–146	80– 92	388–506*	..	..	..	..
Pea Comb	285–262	152–160*	177–173*	..	..	..	..	..
Crest	?	121–113	..	..	..	..	..	..
Polydactyly	161–149	..	..	..	..	..	..	..

* From paper by SEREBROVSKY and PETROV.

TABLE 11

Maximum non-significant deviations from a 1 to 1 ratio expressed in difference between the non-crossover and crossover classes.

N-VALUE	DIFFERENCE	N-VALUE	DIFFERENCE	N-VALUE	DIFFERENCE
150	33	450	57	750	74
200	38	500	60	800	76
250	43	550	63	850	79
300	47	600	66	900	81
350	50	650	69	950	83
400	54	700	71	1000	86

maximum deviation due to chance with varying values of N. The maximum deviation was arrived at by multiplying the probable error by four, thus considering deviations greater than four times the probable error to be outside the range of chance. In order to make the values apply more readily to the data as ordinarily presented, the maximum deviation was doubled so as to express the permissible difference between the non-crossover and the crossover classes instead of the deviation from the mean. Thus in instances where the total number of individuals involved is 350, the non-crossover class may exceed the crossover class as much as 50 and not be indicative of linkage. Where the total is 400 the difference may be as great as 54. Where the *n*-value falls between those two, the significant difference may be estimated. Approximations are permitted here since it is a matter of opinion as to exactly how many times the actual difference must exceed its probable error of the difference.

Table 10 summarizes the results of 35 relationships, three of which (those marked with an asterisk) were taken from the data of SEREBROVSKY and PETROV (1930). It will be noted that the difference between the crossover and the non-crossover classes is significant in the case of rose and pea comb

(SEREBROVSKY and PETROV 1930). However, it is the crossovers that exceed the non-crossovers. This case has been so well established as one of independent assortment that the difference must be due to errors in classification. These three relationships taken from the work of SEREBROVSKY and PETROV were not considered in the writer's investigations. For the nine characters considered, this leaves only one relationship to be tested, crest to dominant white. Accidental loss of stock prepared for this particular study necessitated the postponement of the test of this relationship. Unless the single remaining relationship upon test shows linkage, it may be said that these nine characters show independent assortment. Such behavior is usually taken to indicate that each character has its gene located on a separate chromosome. In other words, each character may be taken as a marker for an independent linkage group. Although the exact number of autosomes in the fowl remains in question, it seems probable that it does not exceed 17 pairs. With only 17 autosomal linkage groups, it is not probable that the first 9 characters chosen at random and completely tested should prove each to be on a separate chromosome. The laws of chance should have given at least two linkages in the 35 tests made. The shortage is even much greater if we consider the number of reported linkages and the total number of tests that have been made.

The sex-linked group of factors in the fowl has been established by the exceptional mode of inheritance of its members rather than through their association. Due to the lack of linkage, several of the members of this group would have been placed in separate linkage groups had they not been identified by means of their mode of inheritance. Loose linkages, or long map distances, may therefore be characteristic of the fowl and account for the shortage of recognized linkage groups. When intervening genes are located, some of the pairs of characters which here have failed to show linkage may later be found to have their genes in the same chromosome. We, therefore, can only state that the nine characters studied all showed independent segregation. Since all possible tests have been made, they may tentatively be considered as markers for independent linkage groups and form a basis for continued studies in this field.

SUMMARY

1. The nine characters naked-neck, rumplessness, white skin, leg-feathering, rose comb, pea comb, crest, polydactyly, and dominant white show independent inheritance. (This excepts the relationship, crest to dominant white, which has not been tested.)

2. Although no linkage was found it seems improbable that each of the above characters belongs to a separate linkage group. The linkage may have been so loose that it was not detectable.

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GENETICS OF THE FOWL. II.
A FOUR-GENE AUTOSOMAL LINKAGE GROUP[1]

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While the study of sex-linked genes in the fowl has proceeded in a very satisfactory manner, geneticists have experienced some difficulty in discovering linkage groups among the autosomes of that species. In this paper there are reported certain data on a case of autosomal linkage which, when considered along with the findings of other workers, permit the definite assignment of four genes to one linkage group and give some idea of their arrangement in the chromosome.

The literature on autosomal linkage in the fowl is so scanty that it may be briefly reviewed. A start in this direction was made when DUNN and JULL (1927) reported some evidence for the linkage of dominant white, cerebral hernia and polydactyly. Additional data presented by DUNN and LANDAUER (1930) made it clear that there is close linkage between the genes for the first two of these characters and suggested that polydactyly might be in the same chromosome but comparatively loosely linked with the other two. More extensive data obtained by WARREN (1932) show independent segregation of dominant white and polydactyly in a backcross progeny of 310 fowls.

Meanwhile SEREBROVSKY and PETROV (1928, 1930) reported linkage of creeper and rose comb, with crossing over of 9.1 percent in 297 progeny from diheterozygous males. A much closer linkage of these two genes was decisively demonstrated by LANDAUER (1931) who found only 0.45 percent of crossing over between them in 4483 backcross progeny.

SEREBROVSKY and PETROV (1930) postulated that creeper also belonged in the linkage group containing dominant white, but their evidence for such an assumption was hardly adequate and the possibility is disproved by the evidence (see table 6) for the independence of dominant white and rose comb. These investigators also present evidence that the genes for blue plumage color and bare neck may be linked.

More recently SUTTLE and SIPE (1932) have reported the finding of about 28 percent of crossing over between frizzling and crest. The data presented below show that frizzling is linked with dominant white. It therefore follows that dominant white, cerebral hernia, frizzling and crest are in one linkage group.

[1] Paper No. 1132 of the Journal Series of the MINNESOTA AGRICULTURAL EXPERIMENT STATION.

It must be pointed out that while a considerable number of linkage trials not reviewed above have shown only the independence of the genes tested, the possibility remains that certain pairs might be in one chromosome but too far apart to show any linkage. Thus WARREN (1932) has presented linkage-test data which, taken with some from the work of SEREBROVSKY and PETROV (1930), cover 35 of the 36 possible combinations in pairs of the following nine autosomal characters: naked neck, rumpless, white skin, dominant white, leg feathering, rose comb, pea comb, crest and polydactyly. All of these 35 trials showed independence. Since the fowl has (probably) 17 pairs of autosomes, one would expect to find by chance two cases of linkage in 35 pairs tested, and the failure to do so does not mean that certain of the pairs of characters may not subsequently be found to belong in one linkage group.

SYMBOLS

The following symbols will be used to designate the pairs of allelomorphs considered in this paper:

I —dominant white	*i* —no inhibitor (of melanin)
h_e—cerebral hernia	H_e—normal skull
F —frizzled plumage	*f* —normal plumage
C_r—crest	c_r —no crest
W—white shanks	*w* —yellow shanks
R —rose comb	*r* —single comb
C —color (melanin)	*c* —recessive white

CROSSING OVER BETWEEN *F* AND *I*

In females. Backcross. Repulsion phase

It has long been known that the White Leghorn is a potentially colored bird in which pigmentation is inhibited by the dominant gene *I*. Fowls heterozygous for *I* are white but usually have a few feathers wholly or partially black. Frizzling has been shown by LANDAUER and DUNN (1930) and HUTT (1930) to be a unifactorial character dependent upon the dominant gene *F*. It is quite useful for linkage studies partly because heterozygotes are quite easily distinguished from both homozygotes and normal fowls, but also because the character is usually recognizable at two weeks or earlier.

In 1930, twelve females derived from a cross of colored frizzled females and White Leghorn male (*ff II CC*) were backcrossed to a White Orpington male. These twelve were thus of the constitution $\frac{Fi}{fI}$ *CC* and the Orping-

ton was $\frac{fi}{fi}$ *cc*. Since the females were homozygous for *C*, the recessive white introduced by the Orpington could not in any way complicate the 1:1 ratio of white to colored fowls expected from the segregation of *I* and *i*. Classifications were made at three weeks and in most cases were checked at four months. Both of the dominant characters are so distinct from their recessive allelomorphs that errors in classification are most unlikely. Red color was introduced by the twelve females, the dams of which were phenotypically frizzled Rhode Island Reds. Although this red is only partially inhibited by *I*, the presence or absence of the latter gene is easily recognized by the absence or presence, respectively, of black pigment in the wings and tail.

In the segregation of *F* and *I* in each of these twelve progenies (table 1) there is evident a distinct departure from the 1:1:1:1 ratio to be expected if the two genes were independent.

TABLE 1

Segregation of F and I in backcross, repulsion phase.

FEMALE	FRIZZLED		NORMAL	
	WHITE *FI*	COLORED *Fi*	WHITE *fI*	COLORED *fi*
K 451	3	6	10	3
K 452	2	10	3	1
K 453	1	7	3	1
K 454	3	5	7	0
K 455	0	5	4	1
K 458	0	4	6	0
K 459	1	4	2	1
K 460	1	6	6	1
K 461	3	5	9	2
K 462	0	3	3	1
K 463	0	4	1	0
K 475	2	2	5	2
Unpedigreed eggs*	2	2	4	0
Totals	18	63	63	13

* Included because all females in the pen were of the same genotype.

While there are 81 frizzled to 76 normal and 81 white to 76 colored, a close fit to the expected 1:1 ratio in each pair, there is a marked excess in the *Fi* and *fI* classes with a corresponding deficiency in the *FI* and *fi* groups. Since *F* and *I* entered the cross in the repulsion phase, the segregation observed is to be expected if these two genes are linked. The birds in all four classes may be grouped as follows:

Parental combinations (*Fi* and *fI*):	126
New combinations (*FI* and *fi*):	31

Crossing over in this case is thus 19.74 percent.

In females. Backcross. Coupling phase

The only data available for this type of cross are those obtained from a mating made in 1932 of females having the genetic constitution $\frac{FI}{fi}$ to an Ancona male having the genotype *ffii*. Unfortunately only three females produced viable chicks so that the data are quite meagre. Classifications were made at two weeks and doubtful cases were re-examined at later periods. The numbers in the four possible classes were as follows:

Frizzled white	*Frizzled colored*	*Normal white*	*Normal colored*
FI	*Fi*	*fI*	*fi*
15	2	4	12

Parental combinations, *FI* and *fi*: 27
New combinations, *Fi* and *fI* : 6

Considering that the numbers are small, the amount of crossing over —18.18 percent—is remarkably close to that found with larger numbers in the repulsion phase.

In the male. Backcross. Coupling phase

In 1932, A 1064, a white male proved to be heterozygous for frizzling and for dominant white, was backcrossed to twelve colored females having normal plumage. Subsequent segregation of *F* and *I* showed that these two genes were carried by the male in the coupling phase, so that the male was $\frac{FI}{fi}$ and the females *ffii*. The progeny were distributed as follows:

Frizzled white	*Frizzled colored*	*Normal white*	*Normal colored*
FI	*Fi*	*fI*	*fi*
39	5	8	30

Parental combinations (*FI* and *fi*): 69
New combinations (*Fi* and *fI*) : 13

The amount of crossing over in the gametes of this male was thus 15.85 percent.

Other evidence of linkage of *F* and *I* was obtained in 1931 from the mating of L 15, a male heterozygous for frizzling, dominant white and color, to five colored females which were heterozygous for frizzling and color. All of these birds were from dams *FfIiCC* in constitution and were sired by a White Orpington. L 15's genetic formula was therefore $\frac{FI}{fi}$ *Cc* and the five females were $\frac{Fi}{fi}$ *Cc*.

The occurrence of recessive whites (*cc*) among the progeny of this cross

would at first sight appear to complicate any reading of the segregation of the *I* and *i* genes, but the theoretical expectations can be easily calculated.

The data are not suitable for the computation of the amount of crossing over, but the observed distribution can be compared with that expected on the basis of the linkage found in other matings. Although crossing over between *F* and *I* in females was 19.74 and 18.18 percent in repulsion and coupling phases, respectively, there is a possibility that linkage may be stronger in males than in females, and accordingly it would seem safer to assume for L 15 an amount of crossing over approximately the same as that found in the gametes of ♂A1064, namely, 15.85 percent. Allowing for 16 percent crossing over between *F* and *I* and the independent segregation of *I* and *C* (BATESON and PUNNETT 1908, HADLEY 1914) the expected proportions of male and female gametes in this cross are as follows:

Male gametes		*Female gametes*	
FIC	21	*FiC*	25
FIc	21	*Fic*	25
fIC	4	*fiC*	25
fIc	4	*fic*	25
FiC	4	..	..
Fic	4	..	..
fiC	21	..	..
fic	21	..	..
	100		100

The theoretical distribution of phenotypes derived from these gametes and that distribution to be expected if there were no linkage are given in table 2 along with the actual frequencies in each class.

TABLE 2

Actual and theoretical distributions of the progeny of L15.

	FRIZZLED		NORMAL	
	WHITE*	COLORED	WHITE*	COLORED
Observed distribution	27	12	3	11
Expected (1) with 16 per cent crossing over between *F* and *I*.	28.2	11.5	4.9	8.4
Expected (2) with independent assortment.	24.8	14.9	8.3	5.0
For (1) $\chi^2 = 1.613$; $n=3$; $P=.66$				
For (2) $\chi^2 = 11.343$; $n=3$; $P=.01$				

* White fowls may be *IC*, *Ic* or *ic*.

By the application of the χ^2 test for goodness of fit it is found that the value of P for the fit to the expectation with independence is so small as to preclude the possibility of independent segregation of *F* and *I* in this cross. On the other hand the P value of 0.66 indicates a good fit to the expectation with 16 percent crossing over. It is recognized that this is only an approximate measure of the amount of crossing over which actually occurred, but the χ^2 tests do eliminate the hypothesis of independence and do indicate linkage.

From all the evidence for the linkage of *F* and *I*, summarized in table 3, it is clear that these two genes are in the same chromosome and that crossing over between them amounts to about 18 percent. The crossing over in a total of 272 measurable gametes is 18.38 percent; but in determining this figure the shortcomings of a result obtained by lumping together gametes of both sexes and having the genes in both the coupling and repulsion phases, are fully recognized.

TABLE 3

Summary of tests for linkage of F and I.

BIRDS TESTED	SEX	TYPE OF MATING	PHASE	GAMETES TESTED NUMBER	CROSSING OVER PERCENT
12 "K" females	♀	Backcross	Repulsion	157	19.74
3 other females	♀	Backcross	Coupling	33	18.18
A1064	♂	Backcross	Coupling	82	15.85
L15	♂	Partial backcross	Coupling	53	Approximately 16.00

ARRANGEMENT OF THE LINKED GENES

From the work of SUTTLE and SIPE (1932) it would appear that the genes for frizzling and crest are about 28 crossover units apart. So far as can be ascertained from their data, it is not clear that all the FfC_rc_r females used in their final crosses had the two dominant genes in the coupling phase. If some of these were $\frac{F\ c_r}{f\ C_r}$ and others $\frac{F\ C_r}{f\ c_r}$ (as seems possible from the fact that these birds were apparently two or more generations removed from the original frizzled, crested parent), the resultant distribution would not give a true measure of crossing over. However, since the percentage of crossing over in these females was almost the same as in a male which was evidently $\frac{F\ C_r}{f\ c_r}$, the data for the females are evidently valid, and it may be accepted that *F* and C_r are about 28 units apart.

This being the case, and the gene *I* being about 18 units from *F*, it follows that *I* and *Cr* should be either closely linked (about 10 units apart) or very loosely linked (depending upon whether *I* lies between *F* and C_r or to the left of both). In the latter case, the crossing over to be expected between *I* and C_r would be 46 percent, less whatever reduction would be caused by double crossing over. The only evidence so far available on this point is found in a small F_2 generation involving *I* and C_r reported by DUNN and JULL (1927) in which the segregation approached that to be expected if the genes were independent. The same authors mention (p. 32) a backcross of an IiC_rc_r male to Brown Leghorn females but report only the ratio of crested to non-crested among the offspring. If *I* and C_r were closely linked, the fact would surely have been noticed in this mating, whereas crossing over of more than 40 percent would have been almost indistinguishable from independent assortment except in larger numbers than they had. It therefore seems probable that *I* and C_r are very loosely linked and that *F* lies between them.

The data thus far available do not definitely establish the relationships between dominant white and cerebral hernia, but DUNN and LANDAUER (1930) have shown that the *I* and h_e are very closely linked. Unfortunately the expression of h_e is somewhat irregular in any but specially selected material so that it was impossible for these investigators to determine more exactly the linkage relations of the genes with which they worked. In one backcross in which the distribution indicates that h_e was fully expressed, crossing over between *I* and h_e was about 11 percent.

In consideration of the findings reviewed above and pending further data, it seems not unlikely that the four genes in this chromosome are arranged in the following order: *I*, h_e, *F* and C_r, and spaced somewhat as is suggested in figure 1. The gene h_e may be on either side of *I*.

It is desirable that some gene be located between *F* and C_r to permit working with shorter map distances in this region, and also that all the existing data be confirmed and extended. Experiments in these directions are in progress at this laboratory.

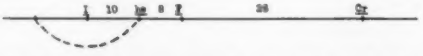

FIGURE 1.—Tentative arrangement of genes in the IFC_r chromosome.

GENES NOT IN THE IFC_r CHROMOSOME

To facilitate further progress in mapping the chromosomes of the fowl it is desirable to report all genes which have thus far been tested and found to show no linkage with those in the IFC_r chromosome. (Students of the genetics of maize have found it preferable to label the chromosomes

of that species by some distinctive gene or genes, rather than as chromosomes I, II, III, etc. For the present, their example seems well worth following.)

Rose comb (R)

In 46 progeny from two *FfRr* females backcrossed to an *ffrr* male the following distribution was found.

Frizzled rose	*Frizzled single*	*Normal rose*	*Normal single*
13	9	11	13

The numbers are small but the class frequencies suggest independence.

Better evidence that *R* is not in the IFC_r group was obtained indirectly by LANDAUER'S (1932) finding of no linkage between frizzling and creeper (table 6). Since creeper is very closely linked to *R* (LANDAUER 1931), the elimination of creeper should automatically eliminate *R* from this linkage group. It is therefore probable that the excess of parental combinations observed by DUNN and JULL (1927) in a backcross of rose, crested males to single, non-crested females (table 6) merely represented a chance deviation from the expected ratio. *R* and *I* were independent in their data.

White Shanks (W)

Evidence that *F* and *W* are independent was obtained in five matings involving these two genes (table 4).

Although the deviations from expectation in the totals for the backcrosses are fairly large, it seems likely that they arose from chance deviations in the progenies of the two males. In these some error may have been incurred by classifying some of the birds before maturity. The dis-

TABLE 4

Segregation of F and W in five matings.

TYPE OF MATING	FRIZZLED		NORMAL	
	WHITE SHANKS *FW*	YELLOW SHANKS *Fw*	WHITE SHANKS *fW*	YELLOW SHANKS *fw*
1. Backcross ♀♀ *FfWw*×♂ *ffww.*	9	8	8	11
2. Backcross ♂ *FfWw*×♀♀ *ffww.*	12	14	12	5
3. Backcross ♂ *FfWw*×♀♀ *ffww.*	2	11	15	7
Totals	23	33	35	23
Expected with independence	*28.5*	*28.5*	*28.5*	*28.5*
4. F_2 observed	24	9	9	2
Expected with independence	*24.75*	*8.25*	*8.25*	*2.75*
5. Partial backcross, ♂ *FfWw*×♀♀ *Ffww*	11	5	2	6
Expected with independence	*9*	*9*	*3*	*3*

tributions in matings 4 and 5 are based on adult classifications and fit the expectations fairly well. More data are desirable but so far these two genes appear to be independent.

This is confirmed by the independent segregation of dominant white and white shanks in reciprocal backcrosses and in a partial backcross (table 5).

TABLE 5

Segregation of I and W in three matings.

TYPE OF MATING	WHITE*		COLORED	
	WHITE SHANKS *W*	YELLOW SHANKS *w*	WHITE SHANKS *W*	YELLOW SHANKS *w*
1. Backcross ♂ *IiWw* × ♀ ♀ *iiww*	17	11	7	8
2. Backcross ♀ ♀ *IiWw* × ♂ *iiww*	10	3	6	4
Totals	27	14	13	12
Expected with independence	*16.5*	*16.5*	*16.5*	*16.5*
3. Partial backcross ♂ *IiWwCc* × ♀ ♀ *iiWwCc*	17	6	14	5
Expected with independence	*19.7*	*6.6*	*11.8*	*3.9*

* In mating 3 white fowls may be *IC*, *Ic* or *ic*.

If the excess in the *IW* class of the backcross progeny were accompanied by a corresponding excess in the *iw* group, linkage would be indicated, but the deficiency in the latter class (table 5) suggests that the excess of *IW* birds can be ascribed to chance or, more likely, to errors in classification. The distribution in mating 3 is based upon descriptions of adults and fits the expectation remarkably closely.

From these two sets of data it is evident that *W* is either not in the IFC_r chromosome, or, if it be present, too far removed from both *I* and *F* to show linkage with either of them.

Other Genes

Evidence that fourteen characters are independent of the IFC_r group is summarized in table 6.

As is to be expected, characters showing no linkage with one member of the group have also shown independence with other members of the IFC_r chromosome. It may be noted that the possibility that polydactyly belongs to this linkage group, as was suggested by DUNN and JULL (1927), is not excluded by the data thus far available. The combined backcross ratio of parental to new combinations when polydactyly was tested with dominant white was 213:186 and the corresponding ratio in trials with crest was 281:243. The question probably will not be settled till some gene is located to the left of *I* in the chromosome, so that the possibility

TABLE 6

*Characters apparently independent of the IFC$_r$ group.**

LINKED GENE	TESTED WITH	INVESTIGATOR	EVIDENCE FOR INDEPENDENCE
I	Recessive white (or black)	BATESON and PUNNETT (1908)	Independence in 77 backcross and in 67 F_2
I	Recessive white (or black)	HADLEY (1914)	Independence in 167 F_2
I	Rumplessness	DUNN (1926)	No linkage in 231 progeny
I	Rumplessness	WARREN (1932)	Backcross 84:104
I	Rose comb	DUNN and JULL (1927)	Independence in 170 F_2
			Backcross 31:28
I	Rose comb	WARREN (1932)	Backcross 88:91
I	Pea comb	WARREN (1932)	Backcross 285:262
I	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 52:37
I	Polydactyly	WARREN (1932)	Backcross 161:149
I	Creeper	SEREBROVSKY and PETROV (1930)	Backcross 96:80
I	Silky	DUNN and JULL (1927)	Independence in 93 F_2
I	White shanks	HUTT (this paper)	See table 5
I	White shanks	WARREN (1932)	Backcross 122:107
I	Leg feathering	WARREN (1932)	Backcross 157:137
I	Naked neck	WARREN (1932)	Backcross 304:308
F	Black	SEREBROVSKY and PETROV (1930)	Backcross 63:50
F	Blue	SEREBROVSKY and PETROV (1930)	Backcross 79:88
F	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 33:40
F	Split comb	LANDAUER (1932)	Backcross 84:91
F	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 24:24
F	Rose comb	HUTT (this paper)	See page 89
F	White shanks	HUTT (this paper)	See table 4
F	Creeper	LANDAUER (1932)	Backcross 97:96
F	Naked neck	LANDAUER (1932)	Backcross 116:117
F	Mesodermal pigment	SEREBROVSKY and PETROV (1930)	Backcross 39:35
C_r	Silky	DUNN and JULL (1927)	Fair fit to independence in 62 F_2
C_r	Split comb	SEREBROVSKY and PETROV (1930)	Backcross 225:203
C_r	Pea comb	SEREBROVSKY and PETROV (1930)	Backcross 177:173
C_r	Rose comb	DUNN and JULL (1927)	Backcross 110:75
C_r	Rose comb	WARREN (1932)	Backcross 80:92
C_r	Rose comb	SEREBROVSKY and PETROV (1930)	Backcross 336:282
C_r	Blue	SEREBROVSKY and PETROV (1930)	Backcross 120:119
C_r	Black	SEREBROVSKY and PETROV (1930)	Backcross 222:215
C_r	White shanks	SEREBROVSKY and PETROV (1930)	Backcross 87:73
C_r	White shanks	WARREN (1932)	Backcross 107:115
C_r	Muff and beard	SEREBROVSKY and PETROV (1930)	Backcross 388:328
C_r	Rumplessness	WARREN (1932)	Backcross 112:122
C_r	Naked neck	WARREN (1932)	Backcross 125:107
C_r	Leg feathering	WARREN (1932)	Backcross 115:119
C_r	Polydactyly	SEREBROVSKY and PETROV (1930)	Backcross 160:130
C_r	Polydactyly	WARREN (1932)	Backcross 121:113

* The ratio given in backcross data is that of parental combinations to new combinations.

of polydactyly being near the extreme left end may be examined, unless the character be first found linked to some other which is definitely known to be independent of this group.

SEREBROVSKY and PETROV (1930) have reported a considerable number of linkage trials involving I, F, or C_r, but some of them had numbers too few to indicate either linkage or independence and for that reason not all of their data are included in table 6. Additional evidence that genes for leg feathering and mesodermal pigment are not in the IFC_r group was provided by DUNN and JULL (1927), chiefly in F_2 data. These workers also found several characters independent of cerebral hernia, but since h_e was somewhat irregular in its appearance in their stocks, and since DUNN and LANDAUER (1930) found it closely linked with I, it seems unnecessary to present their data. Genes independent of I are bound to be independent of h_e.

The association between crest and the trifid or multiple-point condition of the rose comb is well known. In 545 rose-combed birds raised by JULL (1930) from silky crosses, there were only 12 exceptions to the rule that crested birds had multiple point combs and non-crested fowls had single-spike combs. JULL concluded that the twelve had been incorrectly classified and that there is not close linkage between crest and some factor for multiple points but rather that the rose comb is modified by the gene for crest.

THE INFLUENCE OF SEX ON CROSSING OVER

To the best of the writer's knowledge, the first definite evidence that crossing over occurs in the female fowl was found by DUNN and LANDAUER (1930) in a backcross of $\frac{I\ H_e}{i\ h_e}$ females to an $ii\ h_eh_e$ male. This was to be expected in view of the previous finding by CHRISTIE and WRIEDT (1923) that crossing over occurs in the autosomes of the pigeon, which suggested that in birds female digamety is not a bar to crossing over as it is in the silkworm. The numbers observed in either of these cases were hardly adequate for the measurement of the effect of sex on crossing over in birds.

The data thus far available are not sufficient to permit conclusions about sex differences in crossing over in the fowl but it is of interest to note that males thus far tested have displayed slightly less crossing over than females. The figures are:

	Genes	*Crossing over percentage* in ♂♂	in ♀♀
LANDAUER (1931)	C_p* and R	0.19 (2136)	0.68 (2347)
SUTTLE and SIPE (1932)	F and C_r	27.16 (81)	29.87 (77)
HUTT (this paper)	I and F	15.85 (82)	19.47 (190)

* Creeper.

The numbers in parentheses give the number of gametes tested in each case. Pending further data it would appear that the effect of sex on crossing over is somewhat the same in the fowl as in the rat and the mouse. In these mammals crossing over is somewhat lower in the male than in the female.

If further data on crossing over in the two sexes of the fowl should confirm those given above, it would then be apparent that sex differences in crossing over do not depend upon heterogeneity of the sex chromosomes but upon other conditions associated with sex. In this connection it is of interest to point out that in the locust, Paratettix, and the crustacean, Gammarus, crossing over is also lower in males than in females, and that it is not found at all in the male of Drosophila. The absence of crossing over in the female silkworm is apparently at variance with the situation in other species thus far investigated.

SUMMARY

Crossing over between the genes for frizzling and for dominant white was found to be about 18 percent.

This finding along with those of other workers permits the tentative assignment to one linkage group of the genes for dominant white, cerebral hernia, frizzling and crest, and gives some idea of the arrangement of these genes in the *IFC*, chromosome.

It was found that the genes for rose comb and for white shanks are not in this linkage group and the available evidence for the independence of other genes is presented.

Evidence that in the fowl crossing over is less in males than in females is considered and it is suggested that sex differences in crossing over may depend, not on heterogeneity of the sex chromosomes, but upon other conditions associated with sex.

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